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On goes the missile race

Whatever the international contortions of the last few years—the problems of the monetary system, the uncertainties about commodities, the disappearance of growth, the increased vulnerability of governments to non-democratic action—the business of defence has pursued its own self-effacing and growthful way. It is only the annual reviews provided by SIPRI and the International Institute for Strategic Studies (IISS) which remind us that those decisions taken ‘with regret’ a few years ago are now converted to expensive hardware for which there has been little public accountability.

The most recent occasion for us to consider defence expenditure and policy is the appearance of the annual report, *The Military Balance*, of the IISS. It arrives at a most appropriate time, for the failure of the Moscow visit of Mr Nixon to put any more significant teeth in arms control agreements between the superpowers can be assessed in terms of the now likely strategic development of the next few years.

The United States now spends 6.2% and the Soviet Union 5.4% of their Gross National Product on defence. Both percentages have been gently declining in recent years. In the American case, of course, the disengagement from Vietnam has at least checked the rise in military expenditure. In neither case does the strategic weapons programme seem to have been seriously hurt by falling budgets.

● In the United States, the contents of the thousand *Minuteman* silos permitted under SALT are changing rapidly, whilst the silos themselves are being increasingly hardened. Within a year all the 550 planned MIRV launchers will be installed, providing three independently targetable 200-kiloton warheads per launcher. The vast majority of the remainder of the sites already contain *Minuteman 2* vehicles which carry three warheads not independently targeted; there is nothing in SALT to stop the upgrading of all of these to MIRV capability.

● The Soviet Union is constrained to 1,618 silos and is now within 43 of that number. She can have 313 ‘heavy’ missiles (with yields up to 25 megatons) and is close to that total. The remainder are at present mostly megaton SS-9 missiles, but MIRVing is on the way for these too.

● At sea, the United States is permitted 41 nuclear submarines with 710 missiles. At present half of the fleet is MIRVed (each missile containing at least ten 50-kiloton vehicles) but not all the rest will go this way, as ten *Polaris* boats will be pulled out of action in the late seventies and beyond, to be replaced by 24-tube *Tridents*. The warheads in *Trident* are MARVs—manoeuvrable re-entry vehicles, capable of manoeuvre after re-entry to avoid defences.

● The Soviet Navy is restricted to 62 ‘modern’ submarines and is still 20 short of that figure, but building fast. As yet the 660 warheads carried are not multiple vehicles, but tests are under way.

The message is thus from all fronts that the qualitative

revolution is now well advanced. Admittedly the Soviet Union seems to lag two or three years, but for both sides it is becoming plain that accuracy and sophistication is replacing numerical growth in the strategic game. Unfortunately since neither side is able, in its monitoring, to do much more than count devices, the prospects for verified arms limitations (and verifications is probably a *sine qua non*) diminish with time.

A case could be made that these qualitative improvements should be allowed to run their course. After all, if it will be possible to deliver nuclear warheads with an accuracy of a metre by 1990, as some believe, the payload could be reduced and the tradition that the military fights the military and does not draw in the general public might be re-established. There must always be the hope that war could be restricted to surgical strikes.

There are several reasons, however, why we cannot expect to have neat wars fought for us entirely by technology. An obvious one is that there are bound to be more than two participants in any future major war, and since the superpowers, in their pursuit of technological sophistication, have actively, if inadvertently, encouraged other countries to go nuclear but at a less sophisticated level, the multiplicity of nuclear-armed participants will inevitably confuse any bilateral game plan. But there is a more subtle reason why we should not believe that wars of opposing and highly sophisticated technologies will be more bearable for the civilian.

No nation would be satisfied that its future be decided entirely by surrogates. It is unlikely that the idea of a football match, say, between the superpowers—once the shape of the ball had been decided—would be deemed an acceptable way of settling political disputes. There has to be some personal involvement and sacrifice in war, and the growing trend away from this that we see in present and future strategic hardware poses an interesting question. If the early nuclear age has been marked by an uneasy truce between the superpowers because the consequences of major war were unspeakable, maybe the coming nuclear age will see further detente because a technologist’s war between machines having reduced the unspeakable is politically unacceptable.

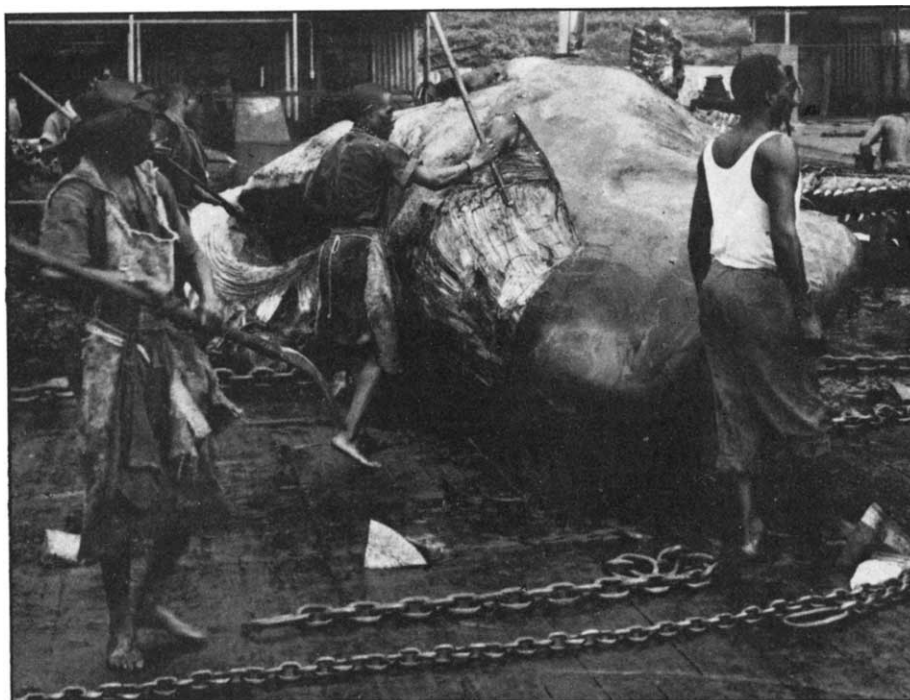
A hundred years ago



WE referred some little time ago to the fact that a sum of about 30,000*l.* had been left to the “London Academy of Sciences.” We hear that already several societies and institutions have sent in, or are thinking of sending in, claims. It is stated, however, that the Royal Society, which certainly is the nearest approach to the institution in Signor Pontif’s mind, has not applied. The Royal Society is of course a mere private body, and might well be held to be justified in refusing to incur the responsibility of distributing a large sum for the furtherance of science; but the miserable chaos in our scientific arrangements is none the less strongly brought out by the present juncture. In England, truly, Science is a body without a head!

From *Nature*, 10, 470, October 8, 1874.





Whales: conserving a resource

In spite of an expression of 'cautious optimism' after this year's meeting of the International Whaling Commission, and a recent Nature article which was optimistic about whale populations, there are still fears that all is not well in the whaling industry. Dr Sidney Holt, Director of the International Ocean Institute in Malta highlights some of the problems still confronting whale conservation and management.

AT the end of the meeting of the International Whaling Commission last June, a number of interested non-governmental organisations put out a statement expressing "cautious optimism" regarding the results achieved there. Dr Ray Gambell has been commendably prompt in bringing these results to the attention of readers of *Nature* (August 9, 1974). We who participated in the IWC meeting must agree that the adoption of what Dr Gambell calls the "amended moratorium proposal" was a significant advance towards rational management of whaling. This is, however, no time for complacency by scientists, governments or the interested public, and Dr Gambell's article is not, I think, sufficiently critical of the present state of whale population assessments. It is not insignificant that Dr Gambell illustrated his article with hypothetical diagrams rather than with an example. "The exact forms of the relationships . . . are not known for certain . . ." is far too mild an expression of real doubts. A number of weaknesses in present calculations are well known to members of the IWC's Scientific Committee, although there seems to be less divergence of opinion within the Committee than in the past. I have drawn the Committee's attention to other deficiencies identified by a special working

party of the FAO Advisory Committee on Marine Resources Research with which Dr Gambell is also associated. I will not reiterate these but merely observe that although they do not all point in the same direction, their general sense is that the IWC's Scientific Advisers may still be erring on the side of optimism. Here, however, I wish to draw attention to some specific points raised by Dr Gambell's article. There is a slip in his exposition of the simple theory of density-dependent rates. The natural mortality rate might "fall as whales are caught", but not because they "are caught before they have a chance to die naturally". In all models used the mortality rates, both "natural" and "due to whaling" are, by definition, independent exponential coefficients, not absolute quantities.

Then there are two significant errors in the table of present sustainable yields (SY) given. SY for female sperm whales in the Southern hemisphere is 1,800, not 4,500. The latter figure is the calculated maximum sustainable yield of females which could be obtained if their numbers were reduced. This would, however, be, in part at least, at the expense of the catch of males, which are far more important because they are both bigger and more numerous. The high quota of 5,000 is designed to reduce the number of females

quite drastically—an act of doubtful wisdom. The SY of 1,700 given in the table for North Pacific female sperm whales is correct (insofar as any of these calculated values are "correct"); the corresponding MSY is 3,400 which is considerably less than the permitted quota of 4,000.

The second error concerns the Minke whale. The stated present SY in the Antarctic of 7,000–12,000 is an estimate of the maximum SY; the Minke is said to be very lightly exploited at present and its present SY is certainly much lower than 7,000. That, however, is not the main point. There are practically no hard facts about the population of this species. As the report says, "The Scientific Committee was unable to reach any conclusion . . . for Minke whales," and it "advised caution in setting catch limits . . ." By analogy with other species, which are rather better known, the Scientific Committee proposed a quota of 5,000, as they did last year. This was not acceptable to Japan and the Soviet Union, who share about equally the present catches, and the 7,000 quota was a compromise; it cannot be described as the outcome of "rational policy". The case of the Minke is important because it has suddenly become, in the last two seasons, an important item in the catches of baleen whales by the pelagic expeditions. From being negligible in the 1968–69 season, it jumped to 12% by weight in 1969–70, and grew to nearly one-third of the total of 190,000 tons taken in the 1973–74 season. The stepping up of the Minke catch is one more step (the last?) in the process of maintaining the profitability of Antarctic whaling by successively exploiting smaller and smaller species of whales—the earlier steps were from blue whales to fin whales to sei whales. That this process is now regulated—but hardly restrained—under a quota system does not mean that it is "rational", that it will ensure survival of a viable population of Minke whales, or that it will necessarily ensure the possibility of high sustained yields from that species in the long term. Judging from past performance, there may be a lapse of up to ten years before the member countries of the IWC are able to agree to act on important advice from its Scientific Committee. Some of the international commissions which regulate the sea fisheries have a similar concept of time and urgency but biologically fishes are more resilient than marine mammals.

It is also a general feature of the history of whaling that by the time there is widespread concern about the survival of one species the industry has already moved to heavy exploitation of another. Thus, while in the past decade or so, "conservation minded" organisa-

tions have become aware of what was happening to the blue whale and, lately, the fin whale, the sperm whale has become by far the most important species both in the Antarctic and in world catches. It now accounts for over 40% by weight of the total catch of all species of whales. Its monetary value is not quite so high because, although sperm oil, the main product, fetches higher prices than, and has different uses from, the baleen oil, the sperm whale meat is not suitable for human consumption. (This means that the argument used by the Japan Whaling Association for continued intensive whaling because whale meat is an important ingredient of the diet of school children and poorer people is not applicable to the sperm whaling industry.) Scientific assessment of sperm whale stocks is especially difficult because of particular features of their biology and of the industry based on them. The IWC Scientific Committee believes that the level of catch of males is at about their maximum sustainable yield in numbers. It is said that a convenient aspect of sperm whaling is that the males are bigger than the females, there is much geographical segregation of the sexes, and the social behaviour (including a "harem" structure) are such that a large "surplus" of males can readily be caught. I find it difficult to reconcile this with the decision to reduce the size limit for females "in order to bring the sexes into better balance". Sperm whales are especially important to the Soviet Union, whose whalers now regularly take over half the world total, yet the USSR delegation to the IWC appeared to be having second thoughts about that decision.

But there is a further problem. Whereas males caught twenty years ago averaged 45 tons each in weight, now they average only 28 tons. The size of females has also declined (though not relatively so much because they have, until now, been less intensively exploited). In fact, the average size of all the large species of baleen whales also has declined over the period in which each of them has been intensively exploited. This means that management for maximum sustainable yield in weight (and hence maximum value of product) requires rather different criteria for management than are at present used. This is particularly true for sperm whales, but for all species the desirable level of population is higher than is needed to maximise the yield in numbers. This should please the whaling industry as well as 'conservationists'. The latter group wants survival of species and of stocks to be assured and for the whales to continue to play an important role in marine ecosystems. The former finds that ex-

plotting stocks when they are at high levels of abundance is much more profitable than when the stocks are depleted. This is as true under management for sustained yields as it is for unrestrained whaling. The only trouble is that to maximise the weight yield from an overexploited stock, one has to agree to more stringent immediate conservation measures, and wait longer for their beneficial effects.

Incidentally, the fact that a substantial part of the sperm whale catch is by ships flying flags of non-member countries of the IWC, that it is not unknown for industries to operate under a convenient flag, especially when international regulations become burdensome, and that some countries refrain from joining the IWC for political rather than technical reasons, suggest that it is desirable for the United Nations Environment Programme and other UN Agencies with wide membership to retain their in-

To maximise the weight yield from an overexploited stock one has to agree to more stringent immediate conservation measures and wait longer for their beneficial effects. . .

terest in whaling, while continuing to assist and support the IWC.

Dr Gambell's article gives the impression that defining MSY in terms of weight involves a new concept requiring special study. On the contrary, apart from being practically universal in fishery management, the MSY (weight) criterion was introduced to the IWC over 13 years ago, at the same time as the attainment of sustainable yields in numerical terms was first seriously discussed there. The necessary data are available, as are the mathematical models; it is simply a question of feeding one into the other. The whaling industry has naturally always measured its success by the weight and price of its products, not by the number of whales caught, and it is time the scientific advisers to IWC did the same.

Calculations of recent and expected yields in weight show that if the 1974-75 quotas for pelagic whaling in the Southern hemisphere are reached (as the 1973-74 ones were not) the catch of all species next season may reach 380 thousand tons, 2 or 3% more than in 1973-74. This pelagic industry is still important; in recent years it has accounted for 60% of the world catch of the larger whale species.

Dr Gambell is right in saying that more research is needed—and at prac-

tically every level, including critical study of the mathematical models currently used, and investigations of the ecological interactions between the species of whales, and between them and other marine organisms on which they feed or with which they compete. In this connection it should be noted that the suggestion to which Dr Gambell refers in his opening paragraph—and it was no more than a hypothetical suggestion—that catching some species may help the recovery of others, is based solely on the fact that they overlap in the composition of their diet. No quantitative evidence whatever has yet been presented to support it. In fact, a number of important assumptions that are regularly made have not yet been tested; these include the assumptions that each whale population is in a steady state before man starts exploiting it, and that his effect on it is merely reversed if and when his predation is eased.

Meanwhile, the world catch of whales is, despite the depletion of the resource and because of the rising prices of meat and oil, still worth about £80 million annually. It could, if stocks were permitted to recover, be worth many times that at present prices—and even more with the expected relative rise in the prices of animal protein in the coming years. Against this we recognise that Dr Gambell and his few colleagues work with very limited facilities and funds, as do similar groups in other countries. Although no whaling expeditions now fly the British flag, the United Kingdom is still a considerable importer of some whale products, and presumably retains a long term interest in resources which could once again yield 10% or more of the total value of the world marine harvest, and practically all from the area of ocean which will be beyond national jurisdictions even if the United Nations Conference on the Law of the Sea agrees eventually to an "economic resource zone" 200 miles wide. Now is the time to make substantially greater means available for research on cetaceans and for international management of them. It would be folly, as some seem to wish, for research to be cut back because of the decline of an important industry, which is a result of our past failure to protect the resource base. At the same time it must be hoped that research on the use of the other living resources of the open ocean, such as the Antarctic krill and the deep water cephalopods, will not be left entirely to the two nations remaining in the business of pelagic whaling. If this is too big an enterprise for other maritime countries, should we not be thinking of a substantial international programme of research and development?

international news

US plans for solar energy

by Colin Norman, Washington

THE US Congress is now putting the finishing touches to a bill which will greatly expand research and development activities aimed at converting solar energy to practical use. Among the provisions contained in the legislation are the establishment of a Solar Energy Research Institute, coordination of solar energy programmes which are now spread over several agencies of the federal government, and the promise of as much as \$1,000 million over the next five years for research and development.

Both the Senate and the House of Representatives have now passed slightly differing versions of the bill, and a House-Senate conference committee was appointed last week to work out a compromise. According to congressional staff members, there should be little trouble reaching agreement, and Congress should be able to send a bill to President Ford to sign before the session ends.

The bill reflects the feeling on Capitol Hill that solar energy has been given too low a priority in the Administration's energy planning, and that present efforts in harnessing sunlight for practical purposes have been too fragmented. It also reflects the fact that a vote for solar energy goes down well back home in election year.

The House and Senate versions of the bill are in complete accord on two major points. First, they would both establish a Solar Energy Coordination and Management Project, consisting of top officials of five governmental agencies and one Presidential appointee, to oversee the federal government's solar energy activities. Such a body "represents a recognition that changes need to be made in the way in which priorities for energy research and development are set and the way in which the Federal Government organises itself to carry them out", according to one of the bill's chief sponsors, Representative Mike McCormack of Washington State. At present, the National Science Foundation has been assigned the leading role in solar energy research and development, but at least a half a dozen agencies have a finger in the pie.

The second chief point of agreement is the need to establish a special Solar

Energy Research Institute. Modelled on one of the laboratories of NASA or the Atomic Energy Commission, the institute would be established at either a new or an existing energy laboratory of the federal government. No determination has yet been made on where the laboratory should be established, but Congressional jockeying can be expected from politicians who would like to have it in their home state.

Both bills also define solar energy to include not only direct conversion of sunlight to electrical power and heating, but also ocean thermal power conversion and windpower.

The chief difference in the bills is the amount of money which they recommend should be spent. A preamble to the Senate version states that solar energy research should be funded to the tune of \$1,000 million over the next five years, and it also provides \$100 million for the remaining half of the 1975 fiscal year. The House version, on the other hand, would provide only \$2 million this year to draw up a research and development, and whatever is necessary in following years. McCormack said during hearings on the Senate bill, however, that the \$200 million a year estimate is probably "in the right ballpark". Whatever the conference committee finally decides, a large increase from the present level of \$50 million a year can be anticipated. □

Research in France: austerity budget

from the Staff of *La Recherche*

WHEN President Pompidou arrived at the Elysee in 1969, he set out to reorganise the structures of scientific policy. The creation of a Ministry of Industrial and Scientific Development in 1969 actually marked a kind of break with the Gaullist period of scientific policy, even though research, for two years, had no longer been given the priority that it had after 1958. This Ministry, which inherited projects from the former Ministry of Industry and those of the former Ministry of Research also had to oversee the Délégation Générale à la recherche scientifique et technique (DGRST) which is (or should be) in charge of the coordination of science policy.

It is this hybrid and rather awkwardly functioning ministerial structure that M. Giscard d'Estaing inherited last May. When the new government was set up, Prime Minister J. Chirac decided not

to modify the structures of scientific policy fundamentally and entrusted the responsibility of a Ministry of Industry and Research (whose projects are almost identical to those of the ex-Ministry of Industrial and Scientific Development) to M. d'Onano. The first problem which confronted the new minister was the preparation of the budget for 1975. Since it was imperative to fight inflation, drastic cuts in public spending for the following year had to be imposed and thus scientific research will have an austerity budget in 1975. After the Prime Minister's bargaining session, the increase in total funds for research and development included in the 'Enveloppe Recherche' (which comprises the largest part of civil research except for telecommunications and major civil aeronautical programmes) will be about 13% in 1975. This budget raise in funds is approximately as large as that planned for all state expenses, but it may be insufficient to compensate for inflation which is currently increasing at an annual rate of about 15%.

Of course, this budgetary growth conceals serious disparities. First of all, one must realise that basic research will be treated to a relatively lesser degree in 1975 than in 1974 since the increase of programme authorisations for the CNRS will be about 7% next year and this agency will be granted very few newly created budgetary positions for researchers: about 100. Only medical research will continue to have relative priority since funds earmarked for INSERM (Institut National de la Santé et de la Recherche Médicale) for equipping its laboratories will be increased by 28% next year. On the other hand the big projects (the so-called 'grands programmes') which have been the object of much controversy for several years in France will undergo an austerity squeeze to a varying extent. Thus, although the budget increase for the CEA (Atomic Energy) is about 16%, that for CNES (Space Research) investments is practically nothing, as are the funds for the 'Plan Calcul', that is computers.

At any rate, in the fields of space and computer science, budgetary decisions are perhaps only temporary. The choices made reflect a certain agreement to make research in the field of energy a high priority item (funds will be increased by 27%). As far as aid allotted to industrial research is concerned, the 1975 budget will mark a

slight acceleration in growth (a 16% increase for the process of aid to development). This research budget, the first of M. Giscard d'Estaing's seven years' term gives some indication of a trend but it does not constitute in itself a scientific policy. The reorientation of research efforts in relation to new priorities implies structural reforms. On this level, major decisions are still being studied. A reorganisation of the Ministry of Industry and Research and of the DGRST could take place in the autumn. The only measure taken in this regard during the summer was to connect the "Delegation à l'informatique" which oversees the 'Plan Calcul' for computers to a new Department of Electronic Industries and Computers within the Ministry. But, at present, the new government's most important innovation is the creation of a Secretary of State to the Universities who is completely autonomous and oversees the universities and the CNRS, thus restricting the power of the Ministry of National Education to elementary and secondary schools. This secretary is expected to outline a policy for university research which the former Ministry of National Education never really concentrated upon. Only during the next few months will one really be able to judge the objectives that the new government intends to pursue in scientific policy. □

Business: problems for Ferranti

from Roger Woodham

ANOTHER British company, the electrical and electronics group Ferranti which makes a wide range of things from naval and aviation electronic equipment to the humble domestic electricity meter, is short of money and has persuaded the government to help out. Looking on the gloomy side and assuming that Ferranti's financial needs continue to grow unabated in the near future, the government has bought the company only a few months of respite with its promise of guarantees to the tune of £5 million so that Ferranti's bank overdraft can be increased yet again. These are the stark facts that make estimates that British industry will be short of perhaps £3,000 million by the end of the year seem plausible.

Ferranti is an interesting case inasmuch as much of its work is inextricably linked up with European defence. Sales last year were worth £72 million, £52 million of that in the British market with 40% being accounted for by defence contracts with the British government. Many of its exports go to Europe but the company describes the destinations of its foreign sales as "traditionally erratic", citing a large one-off contract to supply equipment

to the Brazilian navy last year as an example. In spite of all this, profits have been hard to come by in recent years, for the company as a whole at least; a profit of £3 million in 1967 turned gradually into a loss of £900,000 in 1971 and the accounts are still in the red after two years of improvement.

The development budget—Ferranti says it does no 'research' in the accepted sense—runs at £15 million a year, about a third of which is for governments. The company declines to say how many of its workforce of 17,000 are involved in development because a correlation of money and heads" could be commercially embarrassing.

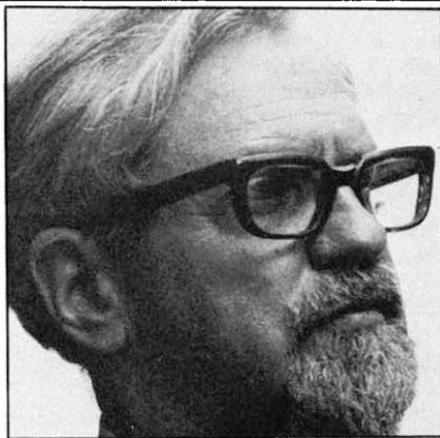
The future for Ferranti in the longer term will depend to some extent on who wins the general election. The present government has several options:

- To buy a stake in the company, thus wresting control from the Ferranti family.
- To bring in an outside organisation to manage the whole concern.
- To provide, as part of a more wide ranging industrial policy, some form of financing for the medium term.
- To persuade the company to sell off parts of itself in order to create a more viable unit. There are several precedents, for example, the sale of Ferranti computer interests to what is now the computer company ICL in the 1960s. □

AMERICA'S first scientifically-based Center for UFO Studies, established by J. Allen Hynek (right) after a wave of UFO reports across the States last year, is planning to ask the National Science Foundation and NASA for funds in the near future. The Center, a loose affiliation of scientists from a dozen US universities, is currently funded by private donations, but feels that its stock in trade—scientific credibility—stands sufficiently high for approaches to be made to establishment bodies like NSF and NASA.

Hynek, professor of astronomy at Northwestern University, Illinois, and author of the best-selling *The UFO Experience*, has been interested in unidentified flying objects for years, and has developed contacts with what he calls the "invisible college" of qualified scientists who believe the UFO phenomenon to be worthy of investigation. In correspondence they agreed that it was time to set up a Center to provide reliable UFO information and to collect reports of events for investigation.

Hynek rented the services of a toll-free telephone switchboard that is manned round the clock, seven days a week, and distributed its number to police throughout the United States. UFO reports on this hot line average



about one a day (or night). Depending on their importance, cases are followed up in person or by a questionnaire.

The scientists of the Center are preparing a report on the 1973 UFO wave in which 1,500 cases are listed. The sightings started in the southeastern states and, in Hynek's words, "spread along the river valleys". When published next spring, the report will be a valuable study of a major UFO event.

Hynek's work has started a steady shift in public and scientific attitudes to UFOs which are documented in a revealing hour-long TV programme being screened coast-to-coast in December by NBC. "I think the programme's

impact will be considerable," says Hynek. "When the next wave occurs the Center will be there to serve."

Hynek is impressed by the fact that the nature of the phenomenon itself is the one thing that has not changed. "The typical UFO report today is essentially the same as it was 10 years ago," he says. Yet he carefully points out that he does not support the idea that UFOs are nuts-and-bolts spacecraft from other worlds: "There are too many things against it. It seems ridiculous that any intelligence would come from such great distances to do reportedly stupid things like stopping cars and frightening people. And there are far, far too many reports."

For Hynek and the members of his "invisible college" there remains no doubt that the UFO phenomenon is real. But its explanation? He draws a parallel with early attempts at explaining what makes the Sun shine, which faltered through lack of knowledge of physics. Currently, all the UFO Center can do is to define the limits of the problem. Says Hynek: "We're setting down the things that any hypothesis will have to explain." And, returning to the conclusion of *The UFO Experience*, he adds: "When the solution does come, it's going to be one hell of a quantum jump." IAN RIDPATH

End of MRC's Edinburgh unit

by Peter Newmark

AFTER seventeen years of pre-eminence in a much-vaunted field of science the Medical Research Council (MRC) Molecular Genetics Unit closed at the end of September. Set up in 1957 at the Royal Postgraduate Medical School, Hammersmith Hospital under the directorship of Bill Hayes, the unit was originally known as the Microbial Genetics Research Unit. In the early 1960s a desire to become more closely involved with teaching as well as research led to negotiations with Edinburgh University. This culminated in a package deal whereby Edinburgh set up a department of molecular biology in which the MRC unit was also to be housed. The chair of Molecular Biology was given to Martin Pollock and his department started up in 1965.

The MRC unit moved up to Edinburgh in 1968 but it was not until 1970 that the department as a whole felt itself to be fully settled down. It was taken for granted at the time of the move that the MRC unit would survive at least until 1978 when Professor Hayes would be 65 and therefore obliged to retire as director of the unit. However in 1971 Professor Hayes began to consider an early departure. It was this that finally precipitated the premature closure of the unit this year.

When the unit was founded microbial genetics was a relatively young subject, but it was clear that it was due for rapid expansion. As such it was an ideal candidate for the MRC to support, in that one of their prime justifications for providing long-term support for a subject is when "for reasons of scale, subject specialisation and duration (it) could not as effectively be organised within a university or medical school". Few people, at least in retrospect, doubt the prudence of the MRC in injecting funds into microbial genetics at that time. The unit rapidly became pre-eminent and began to attract visiting researchers from many countries. It consistently produced very high quality research into the bacterial mechanisms of, for example, chromosome and plasmid replication, transmission of drug-resistance, cell division, host restriction and the control of biosynthetic pathways. It also, in time, achieved a fine record of dissemination of its research interests into British universities. Former members of the unit took up chairs at Kent, Leicester, Newcastle and Sussex.

There is no doubt that the high standards of research emanating from the unit have continued up to the present time. A fall-off in the quality of the unit's research was therefore not the reason for premature closure. It is,

however, hard to define exactly what the reasons were, since it is not MRC policy to divulge them except in very general terms.

The general MRC policy on units of this type is quite straightforward. A unit is set up to encourage a chosen subject on the initiative of an individual who then becomes its director. The planned tenure of the unit comes to the end with the retirement of the director at which time the future of the unit is reviewed. Usually the result is closure of the unit but, sometimes it is continued under a new director. In retrospect at least, it is now clear that the chances that the Molecular Genetics Unit would survive its director were already very slight in 1970/71. The MRC Annual Report for that year stated that "since genetics has now been developed considerably within the universities, the Council no longer feels that there is any general need to renew or establish units (in this field)." As a blanket statement this is not unreasonable. However it is not sufficient as a reason for closing the Molecular Genetics Unit, considering for example that the MRC saw fit to set up the Mammalian Genome Unit in 1973, also at Edinburgh University.

Two main interrelated factors must have been considered by the MRC in coming to their decision. The first was whether there had been a loss of momentum in the research being carried out by the unit. The second was the world-wide shift in emphasis in molecular genetics research from investigating the processes in prokaryotic organisms, especially bacteria, to eukaryotic organisms. The members of the unit dispute that there was a loss of momentum, though they admit that some degree of cohesion had been lost as their individual research interests diversified. They do not dispute that eukaryotic research has become very important, or that it is a suitable field for MRC support, but they are adamant that it would be a great mistake to think that bacterial systems have little left to offer to the advance of molecular genetics.

Although the unit's members are by now prepared to admit that its closure was not unreasonable they remain dissatisfied about the way in which this came about. When Professor Hayes in 1971 first considered retiring from the unit prematurely he immediately enquired of the MRC how this might affect the survival of the unit, at least until 1978. The initial MRC response appeared to be reassuring in hinting that the unit could continue. There was even talk of the likely candidates for taking over the directorship. Professor Hayes therefore settled on his move. Shortly after this and without any consultation with the members of the unit, came the news that a pro-

visional MRC decision had been made to close the unit when the director departed.

As Professor Pollock, who acted as director of the unit after Professor Hayes' departure at the end of 1973, put it: "everyone was dumbfounded". He emphasises that everyone assumed the move to Edinburgh to imply continued long-term support from the MRC. And that had it occurred that this might not extend beyond five years many people, including himself, might not have been persuaded to make the move.

However, at least on paper, the 1971 decision was not specifically directed towards the unit, and, indeed, it heralded two years of discussions before being confirmed in mid 1973. Professor Pollock feels that (at least in the final stages) the handling of these discussions by the MRC was impeccable. Some members of the unit, whose futures were unclear for many months, are less complimentary, although nearly all are happy with the ultimate outcome. The discussions mainly revolved around the fate of the unit's staff. Three of its scientific members already had tenure with the MRC and were therefore at least assured of a job, although in principle they could have been requested to move to any MRC laboratory in the country. However, five senior scientific staff were left with an uncertain future. Professor Hayes decided to put these five up for tenure. The MRC may not have liked this at first but on consideration they encouraged it. Four of the five candidates were successful but again this assured only a salary, not a specified place to work.

The MRC at this stage hoped to disperse the tenured staff throughout the country, with the rationale that dissemination of expertise has a beneficial effect on its recipients. However, this proposal was met with great resistance on the grounds that the collective research capacities of the unit should not be disrupted and that too many personal hardships would be incurred by moving. It is possible that this resistance paid off. In the end, after complex negotiations, many of which involved discussions with the trade unions to which the unit's staff belonged, a solution was achieved. A solution which was unbelievably better than seemed possible in the early stages and which in many ways has the appearance of a face-saving operation by the MRC. All six tenured scientific staff members who wish to stay in the department are to be allowed indefinitely to remain there and do the same research as at present. They are to become members of the University Department of Molecular Biology, with their salaries paid for by the MRC.

In addition the MRC is providing reasonable technical assistance for

By now everybody is aware that the Italian postal services are out of order. The damage to the Italian economy must be enormous, if at all calculable: a typical "transforming" economy which is based on export-import and tourist trade cannot exist without communications. Science, being deeply international by character, must suffer too.

In fact, apart from the usual complaints about the scientific bureaucracy, the most popular topic of conversation among scientists is the abominable state of the postal services. Everybody has a favourite story to tell about some postal disservice, which has been worse than anyone else's. My worst case is the following: an official letter was sent from an organisation to an individual in the same town; in fact the sender and the addressee lives 50 metres apart. The letter took exactly 50 days to arrive: that is, one metre per day.

Officially there is no strike—for that at least one could prepare better—but only a go-slow, so the results are completely unpredictable and haphazard; the fluctuations from the average delay are very large. Apparently all this is caused by the unwillingness of the Ministry to give the men overtime. There are delays the scientists cannot do anything about, and others about which they can. For example, when ordering and receiving scientific materials, the orders go out with many weeks' delay (on top of the ordinary, "inevitable" administrative ones) and the stuff arrives with even greater delay (this is helped by the occasional wildcat strike of the customs). Not only is thus the experimental work greatly hampered, but in these inflationary times it causes unnecessary financial loss.

Scientific journals arrive several months late, if at all (according to newspapers, many tonnes of printed material were simply destroyed by the agents to whom the post office entrusted them for distribution). There is no rational pattern about it. In the Frascati science area there are several libraries subscribing to the same journals; how-

ever, a particular copy might arrive with several weeks' difference and in a random time-order. The March 22 number of *Nature* arrived on the same date (July 12) as that of June 21 in one

Letter from Italy (but only just)



library; or the August 9 number arrived on the same date (September 2) as the August 23 in the next library, 100 metres across the road.

So one is running around the various libraries to find the most up-to-date copy. At least now the average delay is only a matter of a few weeks against several months not long ago. Also one asks one's scientist friends abroad to photocopy a particularly important item and send it by registered express mail. Perhaps it will arrive quicker that way. (Not always: in the spring more than a million registered letters accumulated in a special sorting office near Rome).

Of course, letters are late too—a request for a reprint posted in January in the States, arrived in July. To send out a paper for publication by post is a game of chance. So one tries to exploit people who travel abroad for missions, conferences. In fact, the international organisations here, like FAO and ESRO, as well as large industries, have organised regular weekly courier services, *à la middle ages*. Unfortunately this does not solve the problem of the incoming letters (unless they are addressed to a centre abroad and collected from there).

Particularly vulnerable are scientific meetings in Italy these days. At a recent conference on the magnetosphere in Frascati ten scientists came to the conference whose registrations arrived from one week to three months after the event. About 10% of the participants cancelled their registration, but their letters arrived when the conference was already over. About another 10% did not come probably because of the postal difficulties. One author who could not come sent his paper and his slides by post for presentation at the conference; they arrived one month after the conference. Some of the main speakers did not receive the letter about the division of topics among them: they got to know it for the first time from the printed posters!

Of course, the main message of all this was that 80% of the scientists came nevertheless, delivered their papers, discussed the magnetosphere and other problems while sipping a glass of good Frascati wine. Thus the scientists are not completely downhearted. They help themselves as they can. They use telex for everything possible—or even impossible, like for instance, writing a common paper with colleagues in Boulder, Colorado, via telex. Also, travel arrangements and other urgent matters they conduct over the phone. Because of the numerous overseas calls, the telephone bill has gone up a bit. Letters would be cheaper—but at least the telephone and telex still work. For the moment. **GEORGE MAGYAR**

seven years. This includes secondment of those members of the unit's technical staff who wish to retain the option of continued employment by the MRC. Furthermore like any other research worker, the staff can now apply to the MRC for short-term support of graduate, post-doctorate and visiting research workers. This they have already done with some success. Ironically they might finish up with more MRC funds than the unit was consuming. On the other hand the MRC has more direct control over what research projects they

wish to support in the future.

Are there any lessons to be learnt from the closing of this particular MRC unit? The final result is satisfactory in many ways but inevitably the interim two years generated a lot of anguish amongst those whose careers were threatened. This has certainly confirmed a problem that the MRC were already aware of, namely that they must try and avoid supporting people for so long that it becomes unrealistic for them to find alternative jobs. This has led to a continuing shift in MRC

support away from long-term commitments.

A more specific lesson that has been learnt is that consultation with a unit in future must precede provisional decisions about that unit. With luck this may mean that future closures will involve less suffering. For there is no doubt that two years of indecision has been detrimental both to the research and to the general morale of the ex-members of the late lamented MRC Molecular Genetics Unit. □

THE US Atomic Energy Commission has run into yet another snag with its liquid metal fast breeder reactor (LMFBR) programme. The commission has now acknowledged what has been rumoured for some months—the estimated cost of building and operating a demonstration LMFBR has increased by 250% in two years. The latest estimate, based on a year-long internal AEC study, is that the demonstration plant now being constructed in Tennessee will cost \$1,736 million to build and operate for five years; in 1972, the AEC reckoned it would cost \$699 million. Furthermore, the AEC now says that the plant will not start operating until late 1982 or early 1983, three years behind the original schedule.

These sharply increased estimates, which the commission puts down to inflation and anticipated construction delays, are already causing some government officials to question privately whether the programme should be allowed to proceed at breakneck speed, although at present the LMFBR still ranks as top priority in the Administration's drive to make the United States energy self-sufficient. The argument which is being increasingly raised against the LMFBR is that it is soaking up a huge proportion of the total expenditure on energy research and development, with virtually no short-term benefits. In the last fiscal year, for example, LMFBR research accounted for more than \$400 million in government funds, and according to estimates drawn up by the AEC's chairlady late last year, the entire breeder research programme (including the demonstration plant) could cost nearly \$3,000 million over the next five years.

The issue is likely to come to a head later this year when the AEC finally produces its much-delayed and completely rewritten final environmental impact statement on the LMFBR programme.

● The American Association for the Advancement of Science (AAAS) has been forced to curtail a few activities during the summer in an effort to overcome a severe financial problem—the association ran up a deficit of more than \$370,000 last year, and was probably heading for an even bigger loss this year. Most conspicuous among the belt-tightening moves, which have been imposed by Acting AAAS Executive Officer Philip H. Abelson, is a reduction in the number of pages contained in *Science*, and a decrease in the number of AAAS staff.

Abelson, who took over as Acting Executive Officer of the Association early this summer when the previous occupant of that post, William Bevan, left to take up an academic appoint-

ment at Duke University, described the cut in the size of *Science* as a "painful expedient", but added that the journal "will not be permanently slim". He said that he hopes that it will be possible to wipe the deficit off the books by the end of the year.

The chief reason for the financial crisis seems to be that the association expanded its activities during the past few years at a rate greater than its income allowed. It has, for example, taken on a number of large programmes—such as an effort to improve



Washington seen

by Colin Norman

the public understanding of science—which, although funded by grants, have resulted in large overhead costs to the AAAS. In addition, it cost more than \$150,000 to stage the AAAS annual meeting in Mexico City last year, and the whole situation has been exacerbated by a steep rise this year in printing and paper costs for *Science*. Abelson said, however, that he does not expect that any major AAAS activities will be cancelled.

● These are hard times for people who have a sweet tooth and a weight problem. Not long ago they could sate their appetite for sugar with a choice of artificial sweeteners—cyclamates or saccharin—without risk of the sweetener turning straight to fat. But cyclamates got banned for possibly causing cancer, saccharin stands accused of the same crime (although the jury is still out on that charge) and there is nothing to take their place.

That is why G. D. Searle's new miracle sweetener Aspartame seemed for a moment to be a perfect answer to at least part of the sugar lover's calorie problem. After all, the stuff breaks down into two essential amino acids in the body, it has proved to be safe when fed at moderate doses to rats and dogs and as recently as July it was approved by the Food and Drug Administration (FDA) for a limited number of uses (*Nature*, 250, 259;

1974). What possible damage could it cause? Brain damage, according to one impassioned medical scientist.

Dr John Olney, professor of psychiatry at Washington University School of Medicine in St Louis, has fired off a memorandum to FDA detailing his concern that Aspartame could pose a high risk to children because it may act synergistically with monosodium glutamate (MSG), a food additive which has been found to cause brain damage in mice.

He has asked for a public hearing in which to state his case and the FDA has agreed, although no date has yet been set.

Olney, who has for years been trying to bring public attention to the dangers posed by MSG, believes that the two additives could produce the same toxic effects, and he has some preliminary research results to bolster his case. He argues that the safety margins calculated individually for MSG and Aspartame are both much lower than the FDA claims, and that if the two are taken together, there is no safety margin at all. Furthermore, Olney takes issue with the FDA's contention that a person eating between 1.3 and 1.7 grams of Aspartame a day will be receiving only about 1% of the minimum dose found to be toxic to animals. He points out that since the calculation is based on an adult body weight of 60 kg, a 15 kg child eating the same amount will not have an adequate margin of safety.

Whether or not Olney's claims will be upheld by the public hearing, there is no doubt that the public relations aspects of his charges will seriously tarnish the image of Aspartame.

Meanwhile, in a related development, the FDA refused last month to lift its ban on the marketing of cyclamates. Abbott Laboratories, the sole manufacturer of the sweetener, had petitioned to get cyclamates back on the market, claiming that it has amassed sufficient data to prove that the chemical is safe and that the FDA's 1969 ban was ill-founded. But the agency advised Abbott that its new data is inconclusive and insufficient.

● According to the latest compilation of figures by the National Science Foundation, during the 1969–1975 fiscal years (in other words, during Mr Nixon's Presidency), the proportion of the federal science budget devoted to space activities declined dramatically.

But the proportion spent on defence science and technology has remained constant. At \$10,217 million, the military research and development budget still accounts for more than half the total. □

news and views

Blood disease caused by gene deletion

In normal adult blood the major haemoglobin is a tetramer consisting of two α chains and two β chains. In α thalassaemia, a disease prevalent particularly in south-east Asia, a deficiency in α chains results in the production of abnormal globins—before birth the tetramer γ_4 and in adult life the tetramer β_4 .

The genetics of α thalassaemia are not completely understood but at least two different mutations in allelic genes have been identified, designated α -thal 1 and α -thal 2. α -thal 1 results in a complete absence of α globin synthesis and in the homozygous condition results in Hb Bart's syndrome. γ_4 tetramers are characteristic of this disease which results in intrauterine or early neonatal death. In the heterozygous state, that is, when one gene has the α -thal 1 mutation and one has the α -thal 2, the disease is less severe so that survival into adult life is possible. This condition is known as Hb-H and is characterised by the β_4 tetramer. In some populations (including those in south-east Asia) there are two different structural genes for α globin. The Hb Bart's syndrome follows the loss of all α loci, whereas in the Hb-H disease one out of four loci is functional, presumably allowing a low concentration of the normal $\alpha_2\beta_2$ tetramer.

Weatherall and Clegg (*Br. J. Haemat.*, **18**, 357; 1970) demonstrated that in victims of Hb Bart's syndrome the α chain is completely absent—there being no evidence of abnormal α chains. Since then research interest has centred on the transcription of the messenger for α globin and its translation, to determine whether the α -thal 1 and α -thal 2 mutations reside in the α globin structural genes themselves or in other genes controlling the transcription of the structural genes or the translation of the gene product.

Translation in a cell-free system of the globin messenger RNA isolated from reticulocytes of patients with Hb-H disease reproduces the protein synthesis pattern in the intact cell in that very little α globin is synthesised relative to β globin, suggesting that α globin mRNA is present in very

small amounts of the reticulocytes. The possibility that the cells contained normal amounts of a non-functional α globin messenger RNA could not however be excluded until the experiments by Housman and his colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1809; 1973). Using a probe for α mRNA (prepared by transcribing α globin mRNA into complementary (c) DNA with reverse transcriptase), they clearly demonstrated that the concentration of α mRNA was abnormally low in sufferers from Hb-H disease, and therefore most probably completely absent in Bart's syndrome.

Having established that no messenger RNA for α globin was to be found in reticulocytes of α -thalassaemic patients, the next step in elucidating the molecular biology of the disease was to differentiate between the α structural genes and the regulatory genes controlling the transcription of the structural genes, as the site of the α -thal 1 mutation. This has now been accomplished by two independent groups whose work is reported in this issue of *Nature*: Ottolenghi *et al.* (page 389) and Taylor *et al.* (page 392). Their conclusions are in close agreement. In the conclusive duplicated experiment a cDNA transcript of α messenger RNA was used to probe for complementary sequences in DNA isolated from normal human spleen. The extent of annealing of the probe with normal DNA was about twice that observed for α thalassaemia DNA. The small amount of hybridisation of the α cDNA to the α thalassaemia DNA may be due to contamination of the α mRNA with β mRNA so that the probe contains some β cDNA. Another possibility is that the α cDNA is hybridising to the α -type gene zeta which codes for a globin produced at an early stage in foetal development. Both groups conclude that in α -thal 1 homozygotes all (or a large part) of the structural genes for α globin are deleted from the genome. This is the first time that a gene deletion has been shown to be the cause of a human disease.

PAMELA HAMLYN

Lethal mutants and gene numbers

ONE of the most interesting discussions developing over the past few years has concerned the number of genes in the eukaryotic genome. The apparent discrepancy between coding potential and probable gene number, about which this question is centred, has been most marked in *Drosophila*. That one band might correspond to one gene was first suggested by Bridges¹ because of the apparent equivalence of the cytological and genetic maps of *D. melanogaster*. There are about 5,000 bands in the polytene chromosomes, yet the DNA content of *D. melanogaster* could be divided into 140,000 lengths of 1,000 base pairs (a reasonable estimate for the average length of a gene). Even after restricting the capacity to code for proteins largely to the non-repetitive component of DNA, a concept supported by recent demonstrations that mRNA is derived largely or entirely from this class of sequence, the coding potential of *Drosophila* DNA remains in excess of 100,000 genes.

Any estimate for the number of genes in *Drosophila* thus has immediate implications for the organisation of the

chromosome. At one extreme, if it is of the order of the number suggested by the coding potential of non-repetitive DNA (100,000), all of this component must represent structural genes and there must be many such genes in each band. At the other extreme, a number close to that of the bands would support the earlier apparent equivalence but would bring into question the organisation of DNA within the band and the meaning of the 'gene'. The experimental approach of the geneticist has been to accept the traditional definition of the gene as a complementation group and thus to attempt to define the number of such groups into which known mutants fall.

Mutations in complementation groups must have visible phenotypes to be detected and recessive-lethal mutations are generally used in the genetic analysis of *Drosophila*. In an analysis trying to achieve mutational saturation of the 3A1-3C2 region of the X chromosome of *D. melanogaster*, which displays 15 polytene bands, Judd, Shen and Kaufman² identified 16 complementation groups, each of which was

mapped with deletions to a single band (although with some ambiguity in region 3B where the bands are difficult to distinguish). In subsequent experiments to identify any additional classes of mutant that might have been missed originally, Judd and Young³ isolated mutants that do not alter adult morphology or viability but cause delayed development or female sterility. Two new complementation groups were identified by this analysis, both recessive female-steriles located in the 3B region. The two groups are linked extremely tightly and may comprise a single unit that exhibits intracistronic complementation. Another estimate has been made by Hochman^{4,5}, who has attempted to identify by lethal mutation every gene on the small fourth chromosome of *D. melanogaster*. This chromosome displays 50 polytene bands and thus corresponds to about 1% of the genome. A complementation analysis of 200 mutations resolved 37 loci coding for essential products at which mutations are lethal or cause sterility when homozygous. Another six loci had previously been identified through mutations that are recessive-visible, bringing the total to 43. Estimating the number of probable unmutated loci from the distribution of the 200 mutations in complementation groups suggested the presence of a further 17 genes, bringing the total to 60, slightly in excess of the band number. The number of lethal complementation groups in the 3A1-3C2 X region is thus very close to the number of bands.

Late lethal mutants of *Drosophila* can be identified by a technique developed by Shearn, Rice, Garen and Gehring⁶ for screening third chromosome lethal mutants for those dying after the third larval instar—these mutants are normal in larval functions but defective in functions essential for pupal and adult development. In a complementation analysis of three groups of third chromosome late lethal mutations (72 induced by EMS, 35 by nitrosoguanidine, and 35 by an acridine ICR-170), Shearn⁷ resolved the first group into 60 loci, 55 represented by one mutant each, five identified by two mutants each, and one giving rise to seven mutants (presumably a mutational hot spot); the second group comprised 29 loci mutated once and three mutated twice, the third group constituting 31 loci mutated once and two mutated twice. The Poisson distribution provides a formula which states the relative frequencies of complementation groups not mutated, mutated once, mutated twice, and so on. By fitting the experimental data (ignoring the hot spot) to a Poisson distribution for each of the three sets of induced mutants, the total number of third chromosome genes able to give late lethal mutants was estimated as 364, 172 and 273 respectively. Since 23% of all lethal mutants on the third chromosome are late lethals, this corresponds to a total of 750–1,600 lethal loci; the number of bands on the third chromosome is about 2,030.

If it is true that each complementation group defines a single structural gene (plus any *cis*-dominant regulatory elements), the phenotype of different lethal mutants within each complementation group should be more or less uniform. Shannon, Kaufman, Shen and Judd⁸ concluded that their non-complementing mutants in the 3A1-3C2 X-chromosome region do indeed show similarities of phenotype. Shearn has also found this to apply to the third chromosome late lethals, using a stringent test of uniformity in which imaginal disk developmental potential was assessed by dissection of disks, injection into metamorphosing hosts, and examination for differentiated structures. Disk development is a good criterion since the disks, although superfluous for larval development, give rise to most of the adult structures; many or most late lethals are defective in disk functions. (In an analysis of lethal mutants with imaginal disk defects, Shearn and Garen⁹ recently identified 52 complementation groups on the third chromosome and concluded from a Poisson distribution analysis that there should be at least 435 such loci *in toto* on this chromosome, about a fifth of all complementation groups.) Analysis of the

imaginal disks of each pair of Shearn's non-complementing third chromosome late lethals suggested that in each case both mutants caused the same defect. This is consistent with the idea that each complementation group represents a single gene function.

A critical question which must be resolved before lethal complementation groups can be taken to identify all genes is whether 'null' loci exist—that is, genes whose failure to produce a protein leaves no identifiable mark on the phenotype. Two extreme models are to suppose that null loci are atypical so that the small number of lethal loci identifies most genes, or that lethal loci are atypical and the genome largely constitutes a high number of unidentifiable null loci. An alternative to these models has been discussed by Bishop¹⁰: 'genes' might be multifunctional, each comprising a transcription unit coding for several proteins, so that although null loci might identify single dispensable protein chains in these units, mutation in other parts of the unit would be lethal.

Some apparent null loci have been identified in *Drosophila*¹¹, but before these can be accepted as such it is necessary to show that mutation at each locus represents a deletion—otherwise it remains formally possible that some gene function remains, although in a form not detectable by simple assays for the protein. And assuming that null loci exist, it becomes important to estimate their proportion relative to loci at which deletion is lethal in order to decide whether they represent a major class. But for the presence of null loci to repudiate the complementation group-band equivalence by conferring a protein coding function upon most of the DNA in the *Drosophila* genome, it would be necessary for most genes to fall into this class; although it is clearly reasonable that the absence of some gene products might be compensated by alternative metabolic pathways, it is difficult to believe that most genes may be dispensable.

It is of obvious interest to obtain estimates of gene numbers in other organisms as well as *Drosophila* and this adds to the importance of a genetic analysis of *Caenorhabditis elegans*, a nematode, reported recently by Brenner¹². *C. elegans* is a hermaphrodite (each animal produces both sperm and eggs) and has about 600 somatic cells, half of which are neurones, making it suitable for a planned genetic dissection of the nervous system. In an analysis of the DNA of this organism, Sulston and Brenner¹³ show that the haploid content is 8×10^7 base pairs (about 20 times that of *Drosophila*) and 83% of the DNA sequences are non-repetitive. To achieve a genetic analysis, Brenner has made use of the males which occur in small numbers in a culture and which have the chromosome constitution 5AA+XO instead of the hermaphrodite 5AA+XX.

The 285 mutants, most recessive, induced by EMS and identified by morphological and behavioural differences from wild type, fall into six linkage groups and map at 82 genes. The limited criteria used to identify mutants mean that only one class of genes is represented, but the large numbers of loci at which more than one mutation occurred suggest that mutational saturation of this class was achieved. From the frequency of occurrence of these mutations, Brenner calculates that the average forward mutation rate induced by EMS under his conditions is about 5×10^{-4} per gene. Since the non-repetitive DNA component of *C. elegans* corresponds to about 6.7×10^4 lengths of 1,000 base pairs, if this mutation rate is typical of all genes it would imply that about 34 genes are mutated in each genome. If all the DNA represented essential structural genes, each chromosome would thus carry six lethal mutations linked to the visible mutation isolated—clearly impossible since in this case it should not have been possible to isolate the visible mutant. Another calculation is possible from the measured frequency of induced lethals per X chromosome, of 0.15. At an average induction rate per gene of 5×10^{-4} , this requires 300 genes. This would mean that the six

chromosomes constituting the genome contain less than 2,000 genes, representing only 3% of the coding potential. Each of these 'genes' could contain 30,000 base pairs, a number similar to the average content of the *Drosophila* band. It is interesting to note that the apparent number of lethal loci is of the same order of magnitude as the number of bacterial genes.

Estimates of gene number have also been made for man. Taking the frequency of deleterious mutation at each locus to be 10^{-5} – 10^{-6} as suggested by Muller¹⁴, the human genome of 3×10^9 potential 1,000-base genes should accumulate 3–30 harmful mutations each generation. The unacceptability of such a high genetic load has led to suggestions that only a small part of human DNA codes for proteins. By reversing the argument, if the actual rate of mutation is known, it becomes possible to calculate the number of genes; Ohno¹⁵ has calculated a number of 4×10^4 and another estimate¹⁶ is about 10^5 . But these calculations involve assumptions about parameters such as generation times during evolution and rely upon indirect data for overall mutation rates. Although they are therefore clearly not too reliable, the conclusion that only about 5% of human DNA should code for proteins is in line with the estimates for *Drosophila* and *C. elegans*; if this number is correct, it does not, of course, prove the validity of the genetic calculations, but if much more human DNA codes for proteins, then the genetic estimates must be incorrect.

Each lethal mutation formally identifies an essential site on DNA without making any implication about its function. Some mutations may therefore lie in regulator loci rather than structural genes. But since mutations within a gene or *cis*-dominant control element should fall into the same complementation group, the number of lethal complementation groups can be taken as the number of structural genes. (Of course, any genes coding products which are regulators are included in this number together with genes coding enzymes or structural proteins.) Accepting an estimate of 1,000 base pairs for the length of each gene, these low estimates for gene number imply that only a small proportion of eukaryotic DNA codes for proteins. This conclusion is consistent with recent hybridisation analyses which show that mRNA is derived largely or entirely from non-repetitive sequences^{17–19} and seems to be of comparatively low analytical complexity^{20,21}.

What can be the function of the non-coding DNA? In addition to all or almost all of the intermediately repetitive DNA component, for which a role in providing recognition elements in control systems can be proposed, this class includes most of the non-repetitive DNA component in which the structural genes are located. One extreme model for the structure of the genome is to postulate that each identified lethal complementation group is of a small size, that is of the order of a 1,000 base pair coding sequence and an associated short control element, and that the remaining DNA is without any function identifiable by mutation; if this function is to code for proteins, then most gene products must be dispensable. An alternative extreme model is to suppose that the genome is functionally divided into the small number of lethal complementation groups, each comprising some 30,000 base pairs and therefore including a coding sequence as only a minor part of its length; the purpose of the non-coding sequences can be a matter only for speculation. The apparent correspondence between genetic loci and bands in *Drosophila*, however, which implies that a large amount of non-coding DNA is associated with the coding sequence in each band, fits well with the second model; and biochemical experiments on the sequences within bands therefore offer one of the most promising opportunities to resolve genetic organisation at the level of DNA.

BENJAMIN LEWIN

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Nature and distance of the sources of Vela gamma rays

THE detection of bursts of cosmic gamma rays by instruments on the Vela satellites (Klebesadel, Strong and Olsen, *Astrophys. J. Lett.*, **182**, L85; 1973) has given a much-needed boost to gamma ray astronomy. More than twenty bursts have been observed and they have time scales ranging from one to thirty seconds with evidence of faster pulsations within each burst. So far, no optical or radio emissions have been observed to coincide with the bursts.

One of the fascinating aspects of a discovery such as this in modern astronomy is the speed at which the subject develops; into a few years is compressed what, in previous eras, would have taken decades or even centuries to accomplish. The problem which is occupying the attention of many astronomers at the moment is the question of the distances to the sources of the bursts. As always in astronomy this is a crucial question because, until it has been answered, we cannot estimate the scale of the energy release.

One of the classic tests of the distances to a class of astronomical objects is the distribution of the objects over the sky. This test relies on the fact that our Galaxy is a flattened distribution of stars and gas; thus, provided we can detect a large proportion of any class of objects in the Galaxy, these objects should seem to cluster around the Galactic plane. On the other hand the universe outside the Galaxy seems to be isotropic, and extragalactic objects should be distributed isotropically over the sky. One limitation of this test is that if the objects do lie in our Galaxy, but we can only detect the nearby ones, they will not seem to cluster around the Galactic plane; the naked eye stars are in a class of this type.

An alternative method of gauging the distances to the sources is to measure the distribution in the strengths of the bursts as received at the Earth. If the sources are distributed isotropically in space then the number of sources with an apparent strength greater than any value, *S*,

¹ Bridges, C. B., *J. Hered.*, **29**, 11–13 (1935).

² Judd, B. H., Shen, M. W., and Kaufman, T. C., *Genetics*, **71**,

will be proportional to $S^{-3/2}$. On the other hand if the sources are confined to the Galactic plane the distribution in the apparent strengths will be proportional to S^{-1} ; if the sources are confined to a line as would be the case if they were predominantly in the local spiral arm of the Galaxy, the distribution in the apparent strengths will be proportional to $S^{-1/2}$.

Strong and Klebesadel (page 396 of this issue) have applied these tests to the data they have received from the Vela satellites. The information on the positions of the sources is poor because the angular response of the detectors on the satellites is isotropic; some information is available because each burst was detected on at least two well separated satellites and approximate positions can be derived from the difference in the arrival times of each burst at the two satellites. The distribution in the apparent strengths of the sources seems to be proportional to $S^{-1/2}$ and Strong and Klebesadel claim that this suggests the sources lie in the local spiral arm of the Galaxy. But to draw this distribution, they had to extend their observations to include a number of weak bursts and some suspicion must remain that their data on the weak bursts are not complete.

Many experimentalists will view the conclusions of this paper with some concern. The frequency of the bursts which have been observed by the Vela satellites is small, only ~20 bursts having been recorded in three years. But the detectors on the Vela satellites were small and they were able to detect only the stronger bursts; there was hope that larger detectors flown on balloons would be capable of recording a much greater rate of events which would more than compensate for the shorter exposure provided by balloons. If the distribution of apparent strengths varies as $S^{-3/2}$ then a large balloon-borne detector would record several events per day; but if the distribution varies as $S^{-1/2}$ the rate would be less than one per week and the experiment would be much less attractive.

The lack of reliable distance estimates has not prevented theoreticians from speculating on the nature of the sources. The short time scale of the bursts implies that the dimensions of the sources must be small, and most of the theories which have been proposed have assumed a stellar origin for the bursts. Colgate (*Can. J. Phys.*, **46**, S476; 1968) had predicted that a burst of X rays and gamma rays would be produced by the relativistic shell of material ejected from a supernova explosion; but, according to this theory, the time scale of the burst would be measured in microseconds and not seconds. Moreover no supernova has been observed to coincide with a gamma ray burst.

Stecker and Frost (*Nature phys. Sci.*, **245**, 70; 1973) have remarked that the time structure of the bursts is very similar to that of the X rays which have been recorded from solar flares. The energy output in a typical gamma ray burst, assuming the source is at a distance of 10 pc, is however $\sim 10^{33}$ erg which is more than six orders of magnitude greater than that observed in the strongest solar flare. Stecker and Strong suggest that the gamma ray bursts are flares on magnetic stars which have magnetic fields three orders of magnitude greater than that on the Sun and might therefore be expected to produce much more energetic flares. But it is difficult to see why the time scales should be so similar when the magnetic field strengths are so different.

Another theory, by Pacini and Ruderman, is published on page 399 of this issue. This theory suggests that a burst is produced on the surface of a neutron star when the neutron star suffers a sudden increase in its angular velocity. These sudden increases, or glitches, have been observed in a number of pulsars and it is thought that they are due to sudden decreases in the radii of the stars. A change in the radius of a star would be accompanied by a change in the strength of its magnetic field, and there would be a large release of magnetic energy. Some of this energy would be converted into relativistic electrons which would then radiate X rays and gamma rays. Pacini and Ruderman estimate that there are enough pulsars within a distance of ten parsecs from the earth to explain the observed gamma ray bursts.

Two papers in this issue report the measurement of gamma rays from pulsars. Kniffen *et al.* (page 397) have measured the gamma ray flux from NP 0532 and Albats *et al.* (page 400) have observed gamma rays from PSR 0833-45: It is, perhaps, significant that all the well established sources of gamma rays, apart from the disk of the Galaxy, have intensities which show very rapid variations with time. Bondi and others have stressed the potential which exists in astronomy in the study of phenomena on very short time scales, and it may be in this field that gamma ray astronomy will make its largest contribution.

The stimulus provided in recent years by radio and X-ray astronomy has shown how much the scope of astronomy had been limited when measurements could only be made at optical frequencies; in the future we may see that the long reign of photographic techniques in observational astronomy, and the consequent long integration times of the observations, may have had an equally restricting influence on the development of the subject.

R. R. HILLIER

Historic first with community ecology

from Robert M. May

A MAJOR purpose served by the First International Congress of Ecology, held in The Hague from 8-14 September was to bring to a focus some of the achievements of the International Biological Program (IBP). The motivating idea of IBP is that communities of plants and animals are productive systems with definable inputs and outputs. The hope is that if we accumulate a sufficiently thorough body of measurement and knowledge about these inputs and outputs, we will end up with a better understanding of how ecosystems function, and, in particular, how they respond to the changes wrought by man. Judged on their merits (rather than against the overblown aims which were often invoked to justify the early funding of IBP), the accomplishments are substantial.

Consider, for example, the total annual production of plant material (primary production) of the earth's land masses. Knowledge of quantities of this kind is clearly a *sine qua non* for rational management of the environment. One of the earliest estimates of terrestrial primary productivity is due to Leibig around 1850: 100×10^9 metric ton per year. Ten years ago the widely accepted figure was $40-50 \times 10^9$ ton per year. Drawing together many different strands of investigation, essentially all of IBP origin, H. Leith of the University of North Carolina gave an estimate which is back near Leibig's guesstimate, namely $110-120 \times 10^9$ ton per year. Leith emphasised that there are still uncertainties (another view, put by L. E. Rodin of the Komarov Botanical Institute, Leningrad, and N. I. Bazilevich and N. N. Rozov of the Dokuchaev Soil Institute, Moscow, would assign a more generous figure of 170×10^9 ton per year), but the 110-120 figure seems now secure to within at worst a factor of two. Around 1960 the total marine primary productivity was thought to be about 140×10^9 ton per year; Leith's and the Russian surveys agreed on a current estimate around $50-60 \times 10^9$ ton per year. Thus over the past decade IBP studies have led to almost an order of magnitude revision in the terrestrial/marine primary productivity ratio.

The major remaining uncertainties as to terrestrial primary productivity are in the tropics. These equatorial regions, many of which are under increasing stress from the pressure of human activity, are in need of much further research. Leith and others emphasised that temperate zone agricultural systems are unlikely to be best

for tropical and subtropical regions.

Reviews of current knowledge about secondary production—microbial and faunal production in terrestrial, freshwater and marine ecosystems—were given jointly by O. W. Heal from Merlewood Research Station, England, and S. F. MacLean, from the University of Alaska, and also by A. Macfadyen, from the New University of Ulster. These reviews, which included discussion of the comparative productive efficiencies of different classes of organism and of different levels in the trophic chain, were necessarily painted with a coarse brush, as comprehensive data are rare for most types of ecosystem and much of the IBP research remains to be digested. Overall, there emerged estimates of the total secondary productivity in various types of ecosystem which seem secure to within a factor of ten. In particular, there was general agreement that we are beginning to understand the limits to the rate at which material can be processed as it passes along the food chain; but there can be major upsets and fluctuations from year to year (as, for example, in pest outbreaks or lemming cycles).

One of the more noteworthy conclusions of the studies of secondary production is that the bulk of it takes place below the ground. The birds and bees may engage scientific curiosity, but 90 to 99% of secondary production is by decomposer organisms in the soil and leaf litter. MacLean humorously underlined this point by remarking that if humans must consume at the secondary rather than exclusively at the primary level (eat meat instead of only plants), they should at least consider eating earthworms rather than beef.

In the plenary session devoted to "diversity, stability and maturity in natural ecosystems", G. H. Orians of the University of Washington, R. H. Whittaker of Cornell University and R. M. May of Princeton University marshalled various lines of empirical and theoretical evidence to suggest that communities with a rich array of species and a complex web of interactions (the tropical rainforest being the paradigm) are likely to be more fragile than relatively simple and robust temperate ecosystems. This inverts the conventional wisdom that "complexity begets stability", but seems more in accord with such reported facts as: (1) relatively simple arid ecosystems, including human populations with different economies, have persisted for long periods, fluctuating within well-defined limits (I. Nor-Meir, Hebrew University, Jerusalem); (2) estuarine ecosystems seem to be disturbed less by, and to recover faster from, indignities imposed by man than do marine ecosystems of

higher diversity (D. F. Boesch, Virginia Institute of Marine Science); (3) the tropical rainforest seems to be a 'non-renewable source' when it is disturbed over sufficiently large areas (A. Gomez-Pompa and C. Vázquez-Yanes, Institute of Biology, UNAM, Mexico).

The final two days centred around "diversity, stability and maturity in ecosystems influenced by human activities", and "strategies for management of natural and man-made ecosystems". Here most speakers agreed that many aspects of human activities are now on a scale comparable with natural biogeochemical processes, but that both short and long term impacts are uncertain. N. Alexander of Cornell University discussed the response of microbial communities to human activities. He pointed out that microorganisms are of enormous importance to the functioning of natural communities, that the impact of pesticides, polychlorinated biphenyls, acidic mine wastes and suchlike on various populations of microorganisms cause disturbances which range from "none to catastrophic", and that our ability to predict such outcomes are as yet woeful. In a major review paper, J. Jacobs of the University of Munich noted the ambiguities and uncertainties associated with the definition of human impact on

natural systems, which are typified by a study of bird biomass and diversity in Finland: in Helsinki, there were 21 species and a biomass of (in some appropriate units) 1,089; in rural areas near houses, an average of 80 species and a biomass of 371; in areas undisturbed by man, an average of 54 species and a biomass of 297.

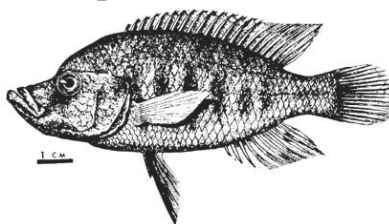
The above account touches upon some of the larger themes of the congress. In so doing, it unfortunately has to pass over many interesting individual contributions. I particularly enjoyed the account by M. Cody, of UCLA, of the parallel and convergent evolution of the avifauna in 'Mediterranean' habitats in California, Chile, and Africa. This study was part of the IBP Convergent Ecosystems Project, and it provided detailed illumination of the way species with different evolutionary histories come to have similar roles, to provide striking constancy in the ecological structure of geographically separated communities. A diverse array of skills was brought to bear by G. H. Orians, R. G. Cates, D. F. Rhoades and J. C. Schultz, of the University of Washington, to elucidate some aspects of the cost-benefit accounting of the biochemical defences which plants maintain against the beasts which seek to eat them, and of the biochemical strategies in turn adopted by these consumers.

I think the congress would have been improved by widening its scope in two respects.

By focusing on the structure and function of whole communities of plants and animals (community ecology). For instance, theoretical notions about domains of stability, or about some numerical characterisation of ecosystem 'resilience', are beginning to be brought into confrontation with the real world in recent studies of low-dimensional systems such as insect host-parasite populations; for complex multispecies communities I think such theoretical speculations are a long way from quantitative testing against nature.

Moreover, the congress was largely confined to technical aspects of how ecosystems work. Given that the word 'ecology' has come to mean all things to all people, such a narrow construction was probably the wisest course for a professional meeting. Even so, I would have liked to see one looser session, in which economists and professional ecologists aired speculations as to whether, for example, the contemporary global patterns of inflation are tied to real diminution in *per capita* supplies of food and energy, or whether these patterns are merely artifacts of present economic systems. To have held such a session may have run the risk of vulgarising the congress, but to have done otherwise runs the larger risk of seeming to fiddle while the world burns.

Indigenous fish



Haplochromis parorthostoma, found only in Lake Victoria, East Africa. It is one of more than 100 endemic species of the cichlid genus *Haplochromis* which have evolved in the 750,000 years for which the lake has existed. According to P. H. Greenwood (author of *Cichlid Fishes of Lake Victoria: The Biology and Evolution of a Species Flock*; British Museum, London, 1974, £6.00) the explosive speciation was the result of a fortuitous combination of circumstances. Lake Victoria (originally a group of lakes) offered many environmental niches; the extent of anatomical specialisation of the ancestral river fish was neither too great nor too small to allow further specialisation in Lake Victoria; and the complexity of the fishes' breeding behaviour allowed barriers to interspecific crosses to evolve.

Is the Martian core solid or liquid?

from Peter J. Smith

FROM the mass, radius and moment of inertia of Mars as determined by orbiting and passing spacecraft, it may be reasonably concluded that the planet has a central core. But is the core solid or liquid? The correct answer to this question is not only of interest in its own right; it may also help to resolve a disagreement which is about to arise over whether or not Mars possesses an internal magnetic field. Last year, Dolginov *et al.* (*J. geophys. Res.*, **78**, 4779; 1973) interpreted Mars 2 and Mars 3 magnetometer data in terms of just such a field; but apparently Bogdanov and Vaisberg will shortly argue that the observed magnetic field variations are the result of solar wind-planetary ionosphere interaction instead. Clearly, if it could be shown that the Martian core is solid, an intrinsic magnetic field (at least, produced by dynamo action) would be ruled out, although it must be admitted that proof of a liquid core would fail to resolve the issue either way.

In a situation where the confirmed presence or absence of an internal magnetic field cannot itself be used to infer the state of the core, the obvious approach is to examine the thermal conditions in the Martian interior. Unfortunately, there are no experimental thermal data from Mars. Mariner 9 revealed evidence of extensive volcanism (including a series of prominent shield volcanoes), mildly cratered plains similar to the lunar maria, and circular features such as domes and craters; but although this suggests that at some time in the past Mars must have been hot enough for partial melting to have occurred at depth, it can give no sure indication of present conditions.

In an attempt to deduce whether the Martian core is solid or liquid, Young and Schubert (*Geophys. Res. Lett.*, **1**, 157; 1974) have therefore constructed a thermal model of Mars which involves no experimentally determined thermal boundary conditions but draws on experience and information gained from the application of similar techniques to the Earth. Indeed, as far as structure is concerned the model is a straight 'Earth model' with core, mantle and crust separated by rigid boundaries. The core itself is assumed to contain no heat sources (so there is zero heat flux at the core-mantle boundary), but heat sources are distributed uniformly throughout the rest of the planet (so temperature and thermal gradients are continuous across the crust-mantle boundary). The crust, whose outer boundary is taken to be an isothermal surface, is only able to conduct heat;

but the mantle is a region in which solid-state thermal convection is an important mode of heat transfer. It is this last characteristic which particularly distinguishes the Young-Schubert model from most previous thermal model studies of planetary interiors.

In numerical terms, two sets of core parameters are examined: an iron core with a radius 0.4 of the total Martian radius and mass 0.1 of the Martian mass, and a pure FeS core of radius 0.6 and mass 0.26. These core models are the maximum density-minimum size and maximum size-minimum density cores consistent with observed mass, radius and moment of inertia of Mars. The radioactive heat source concentration in the crust-mantle is taken to be the same as that in the terrestrial mantle (2.61×10^{-7} erg cm⁻³ s). The thickness of the conducting crust will be determined by the depth at which the conduction solution for temperature rises to about 1,000° C above the surface temperature of the planet, and is thus highly dependent on the chosen value of heat source concentration. But crustal thickness should be at least 100 km because a thinner crust would probably be subject to plate tectonic activity, whereas no evidence of such activity is available from the Mariner 9 investigations.

The most important point to emerge from the model calculations is that the thermal convection in the mantle is remarkably successful in reducing temperatures (as in the Earth), thus vindicating its inclusion in the model in the first place. For a pure iron core, for example, a temperature difference of 8,000° C between the core-mantle boundary and the planetary surface when there is no convection is reduced to about 2,300° C when convection occurs at a Rayleigh number of 100 times the critical value for the onset of convection. The actual Rayleigh number is, of course, uncertain because viscosities are not known; but one certain point arising from the models is that, for pure conduction, temperatures rise above typical silicate melting temperatures. The implication here, therefore, is that convection in the Martian mantle is actually necessary to prevent large-scale melting there.

And because viscosities are unknown, it is not possible to deduce absolutely whether the Martian core is solid or liquid. But what can be said is that if viscosity is lower than 10^{22} – 10^{23} cm² s⁻¹ at temperatures above about 1,500° C, mantle convection will then be efficient enough to cool the core-mantle boundary to temperatures below those at which Fe or Fe-FeS cores would be liquid. As several workers have concluded that Martian viscosity is indeed below this limit, the Young-Schubert model suggests that a dynamo-produced

magnetic field in the Martian core is not possible.

Finally, one other important conclusion to be drawn (specifically from the model temperature profiles) is that if partial melting occurs at all in the mantle it will do so at depths of about 300 km. By analogy with the Earth, this suggests that the Martian lithosphere would be several hundred kilometres thick, or intermediate in thickness between those of the Earth and the Moon.

Gravitational waves from collapsing stars

from P. C. W. Davies

ALTHOUGH it is becoming increasingly likely that Professor Joseph Weber's longstanding attempt to detect gravitational waves from a cosmic source has not after all met with the spectacular success originally claimed, the stimulus that his work and propaganda has given to theoretical studies of this phenomenon continues to operate unabated. Gravitational waves are predicted to occur whenever massive objects are disturbed, much as electromagnetic radiation is produced by accelerating charged particles. The main difficulty in the detection of the former rather than the latter is the incredibly low cross section of ordinary matter to these disturbances. Consequently, only very massive and violent cosmic events occurring in relative proximity to the Earth are capable of producing a pulse of gravitational radiation sufficiently energetic to be detectable with current technology.

Attention has traditionally been given to three possible cosmic sources of such radiation: violent agitations occurring in the primeval fireball of the big bang, objects crashing into black holes and the explosion or collapse of stars. Most books on gravitation contain order of magnitude estimates of the strength of the ensuing radiation, but few explicit numerical calculations seem to have been published. A welcome contribution to the physics of gravitational wave pulses from collapsing stars appears in the August 1 issue of *Astrophysical Journal* (**191**, L105–L107; 1974). Detailed calculations have been performed by Thuan and Ostriker of the Princeton University Observatory for a number of simple scenarios of the collapse of a white dwarf star into a neutron star or black hole.

It is well known that there can be no gravitational radiation from a spherically symmetrical collapsing object. A real star will, however, possess asymmetries, and these tend to become amplified during the collapse process.

The authors therefore consider a uniformly rotating star of uniform density and perform a calculation of the energy radiated away in the form of gravitational waves during the collapse to lowest order approximation in the gravitational potential. This sort of collapse is known to occur for a white dwarf star with a mass a little in excess of the mass of the sun. Calculations based on Newtonian theory show that a spheroidal shaped object (such as might be the shape of a rotating white dwarf) becomes more oblate as the collapse proceeds, until finally, after a finite period of time, it assumes the unlikely shape of a zero thickness pancake at a minimum radius, say $r_{\min}$. The Newtonian approximation will not be valid if $r_{\min}$ becomes comparable with the Schwarzschild radius r_{sch} .

Thuan and Ostriker first find that a non-rotating spheroid of mass M which starts at rest at infinity with infinitesimal eccentricity, emits a pulse of gravitational radiation of total energy $37 \times 10^{-3} (r_{\text{sch}}/r_{\min})^{7/2} Mc^2$ erg. This result should be compared with the analogous case of two point masses collapsing in from infinity from an initial state of rest which liberates only $1.2 \times 10^{-3} (r_{\text{sch}}/r_{\min})^{7/2} Mc^2$ erg. Evidently spheroidal collapse is extremely efficient for the production of energy in this form.

Next the authors include the effects of rotation. The highest output would occur in this case if the star was very

rapidly rotating with a large ratio of initial to final radius. Under these circumstances the energy radiated is boosted further to $109 \times 10^{-3} (r_{\text{sch}}/r_{\min})^{7/2} Mc^2$ —a gain of 91.6 on the value for two collapsing point masses. Moreover, as the authors point out, rapidly rotating stars are unstable to the growth of non-axisymmetrical perturbations, such as might be produced in reality by an oblique magnetic field. These additional disturbances would increase the radiation emitted still further. Physically, the reason why rapidly rotating collapsing stars radiate so much energy away in gravitational waves is partly due to the way in which the rotation delays the collapse of the equatorial parts of the star in a region of high gravitational potential during which time the poles quickly collapse inwards, so flattening the object still further.

Turning their attention to a realistic model of a white dwarf, the authors then estimate the energy emitted during the collapse of a $1.4 M_{\odot}$ Maclaurin spheroid with uniform rotation and uniform density of 10^9 g cm^{-3} . An upper limit to this energy turns out to be about 10^{32} erg, and a lower limit around 10^{45} erg. A single pulse of this sort will have Fourier components nearly constant over a wide frequency range, after which they fall rapidly.

In practice a collapsing white dwarf might well emit a whole series of pulses as it 'bounced' back and forth through the pancake configuration, finally

settling down into a neutron star. However, assuming a Weber-type experiment attempts to detect a single pulse of the type considered, even under the most favourable circumstances the collapsing star would still need to be as close as 100 light years for any hope of success

The changing micrometeoroid influx

from David W. Hughes

THE disparity between the flux of micrometeorites at 1 AU obtained from lunar microcrater counts and from satellite data has been known for many years. A typical example is shown on page 380, the satellite data coming from the work of Alexander (Baylor University, Waco, Texas), the data from the study of microcraters on the surface of lunar rocks and spherules coming from the work of Neukum and Schneider (Max-Planck-Institut für Kernphysik, Heidelberg). In the 10^{-10}g region the two techniques give results which differ by a factor of 200 and it is of great importance to decide if this difference is due to some physical cause or is just an unresolved systematic error in one or both techniques. Fortunately the influx of meteoroids to the lunar surface is the same as that to the Earth's upper atmosphere (most of the satellite observations are made in near-Earth space), the circumterrestrial dust cloud, thought in the late 1960s to exist, now being discounted as a figment of incorrect calibration. But satellite-borne detectors do measure the present day influx, whereas lunar microcrater counts measure the mean influx over the last few tens of thousands of years.

To convert the crater population on a given lunar sample into an interplanetary dust flux the size and form of the craters have to be related to the mass of the causative meteoroid. One immediate problem is that crater size also depends on meteoroid velocity, density, and shape. Up to now the meteoroid impact velocity has been thought to be about 20 km s^{-1} . Two recent results indicate that it is much lower. Doppler shift observations of the solar spectral lines scattered by the solar system dust cloud give a geocentric velocity of about 5 km s^{-1} and this observation has recently been backed up by measurements of microcraters found in glass, steel and pyroxene surfaces exposed for 46 days during the Skylab mission. Nagel, Fechtig, Schneider and Neukum (Max-Planck-Institut, Heidelberg) reported these observations at the recent COSPAR Meeting (Sao Paulo, 1974). They compared the spall zones, smoothness of the central depression, fracture damage and general shape of the 47 observed craters (diameters $>1 \mu\text{m}$)

Still no gravitational radiation

by John Gribbin

LEVINE and Garwin report in the latest issue of *Physical Review Letters* (33, 794–797; 1974) a new negative result for the detection of gravitational radiation pulses, at a level six times more sensitive than the experiments they reported last year (*Phys. Rev. Lett.*, 31, 173–176; 1974). They say that they believe "the present results to be in substantial conflict with the detections reported by Weber".

The improved sensitivity over earlier results from the same group has been achieved chiefly through increasing the mass of the aluminium cylinder used as an antenna from 120 kg to 480 kg; the resonance frequency is now 1,637 Hz, close to that of Weber's apparatus, 1,661 Hz. During the 27-day period December 3 to December 30, 1973, only one measurement out of 4.1×10^7 values obtained for the mean oscillation energy kT contains a pulse substantially above the noise level.

According to Levine and Garwin, that pulse could be a

gravity wave pulse, stress relaxation in the antenna, electrical pickup of "an intense but infrequent laboratory tone", or a signal-processor defect. Since no coincident pulse was observed at the University of Rochester detector, the gravitational radiation explanation seems the least tenable.

Further analysis of the data—including Weber's observations as far as they are available—seems to rule out both the possibility that gravity waves were detected in May 1973 but the pulses have not been produced since then, and the possibility that "hundreds of gravity waves were incident per day even during December 1973, giving our bar $<0.3 kT$ ". In addition, Levine and Garwin note that an error in the computer program used by Weber's group for some of the analysis would have produced a spuriously high number of pulses, and that Weber has acknowledged the existence of this error. All in all, it seems that gravitational radiation has not yet been detected.

with the form of craters produced by simulated micrometeorites, these particles having been accelerated using a Van der Graff accelerator to known velocities in the 1 to 20 km s⁻¹ range. In many cases these characteristics indicated the relatively low impact velocity of about 7 km s⁻¹. So both the curves in the figure have to be moved to the right by factors varying with the velocity dependence of microphone response, penetration hole size and crater diameter. As these effects are functions of meteoroid momentum or kinetic energy the use of this new velocity determination is still not sufficient to bring the curves together.

Two other things are assumed when dealing with microcraters, first that the ratio of the diameters of the microcrater pit and the impacting particle η is 2 and second that the density of the particle is 3 g cm⁻³. Both these assumptions need to be reconsidered; recent laboratory experiments have shown that η decreases with particle density and also that the mean particle density varies with mass. Both these factors make the interpretation of microcraters more difficult. Measurements of crater circularity have led Brownlee (University of Washington) *et al.* (4th Lunar Science Conference, Houston, 1973), to the conclusion that the majority of microparticles have equidimensional shapes and that shallow craters are produced by oblique incidence so the direction of the incident flux has also to be taken into account.

The main problem however lies in estimating the time the rocks under consideration have remained exposed on the lunar surface. One way of doing this is to count the tracks in the rock surface produced by retarding solar flare particles. Knowing the present day flare flux and assuming that solar activity has remained constant for about 10⁶ years gives the exposure age. This second assumption is the stumbling block; the data of Neukum and Schneider could indicate that either a lower particle flux existed in the past or

that the solar flare flux was higher then than now—both would produce the same result.

McDonnell, Ashworth, Flavill, Bate-man and Jennison (University of Kent) overcome this problem by considering surfaces with an equilibrium distribution of craters. Laboratory simulation indicates that solar wind sputter erosion (0.043 Å yr⁻¹) is the dominant factor determining crater lifetime. Knowing this erosion rate enables the micro-particle influx to be derived from the equilibrium distribution. The authors conclude (COSPAR, Sao Paulo, Brazil) that the microparticle influx has increased by a factor of four in the last 10⁵ years.

This would account for the discrepancy shown in the figure but creates the question of why this flux has increased.

Adaptations of plants to drought

from Peter D. Moore

THE C₄ dicarboxyl acid pathway of carbon fixation has now been recognised in a wide variety of plant species from at least six orders of flowering plants. In this process CO₂ combines with phosphoenolpyruvate and is transported to the bundle sheath cells in the form of dicarboxylic acids, where it is released as CO₂ to be fixed by the conventional Calvin (C₃) system. The widespread occurrence of the mechanism suggests that it arose independently in more than one order (Evans in *Photosynthesis and Photorespiration*, edit. by Hatch, Osmond and Slatyer, 130, Wiley, New York; 1971). This being so, it has been difficult to study the evolutionary development of the process. Yet the work of Bjorkman *et al.* (*Yb. Carnegie Instn Wash.*, **68**, 620; 1969 and **69**, 640; 1970) indicates that very few genes are involved.

Troughton (in *Photosynthesis and Photorespiration*, 124; 1971) has proposed that a study of the isotopic ratio of ¹³C to ¹²C (this is normally expressed in relation to a standard and is termed $\delta^{13}\text{C}$) in plant tissue should indicate the relative importance of the two systems of carbon fixation at the time when the plant was alive. This is because C₃ plants discriminate more effectively against ¹³C in their carbon uptake than do C₄ plants; as a result C₃ plant tissue has an average $\delta^{13}\text{C}$ of -29‰ (that is proportionately less ¹³C than in the air) whereas C₄ plants' average ¹³C is -14‰.

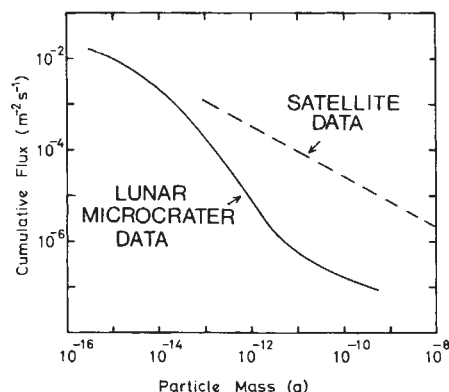
Troughton, Wells and Mooney (*Science*, **185**, 610; 1974) have now used this technique to study the relative importance of the C₄ pathway over the past 40,000 years in the C₄ desert plant *Atriplex confertifolia*. Two ancient

middens of the pack rat *Neotoma* from the Mohave Desert region of Nevada yielded material of this species which dated from >40,000 yr (beyond the scope of radiocarbon determination) and 10,000 yr respectively. The $\delta^{13}\text{C}$ of the older sample is -13.4‰ and that of the younger is -16.2‰. A modern sample of *A. confertifolia* yielded a $\delta^{13}\text{C}$ of -14.8‰. Thus this species has been using the C₄ pathway for more than 40,000 yr.

Troughton *et al.* also determined the $\delta^{13}\text{C}$ of *Opuntia polyacantha* tissues from the same sites. This species is a desert succulent which also concentrates carbon dioxide before fixing it by the conventional mechanism, but this time by crassulacean acid metabolism in which malic or citric acid concentrations build up during the night and the carbon accumulated in this way is subsequently used in the Calvin cycle. Once again these crassulacean acid (CAM) plants do not discriminate as strongly against ¹³C as normal Calvin plants, and their $\delta^{13}\text{C}$ values resemble those of C₄ plants. In *Opuntia polyacantha* the >40,000-yr-old material gave a $\delta^{13}\text{C}$ value of -21.9‰, and the 10,000-yr-old sample gave -13.9‰. This suggests that the degree to which *Opuntia* depended upon the CAM technique of carbon concentration increased over the time interval between the two samples.

It is known that CAM plants become more dependent on night-time accumulation of carbon for their photosynthesis and growth under conditions of water stress. Indeed this mechanism can be regarded as an adaptation to dry habitat conditions in which normal daytime photosynthesis renders a plant liable to desiccation. The change in the $\delta^{13}\text{C}$ values of the *Opuntia* tissue analysed may, therefore, reflect the changing contemporary climatic conditions in Nevada. Other plant remains found in the area support this suggestion, since they give evidence of pluvial juniper woodlands, corresponding to the Wisconsin glaciation of the north, giving way to arid desert at about 10,000 yr b.p.

There are similar indications from the *Atriplex* material. C₄ plants generally and *Atriplex confertifolia* in particular are frequently associated with rather dry habitats; their cellular resistance to CO₂ exchange is apparently lower than in C₃ plants, which may account indirectly for their capacity to withstand drought. It is significant, therefore, that the remains of *Atriplex* are considerably more abundant in the 10,000-yr-old specimen than in the 40,000-yr-old one. Thus palaeontological and palaeobiochemical evidence provide complementary information concerning the climatic changes during this period.



Number of micrometeoroids, with mass greater than the given mass, which will impact every second on a square metre of exposed, surface one astronomical unit from the Sun.

articles

Proterozoic crustal distribution, mobile belts and apparent polar movements

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The geological significance of a Precambrian supercontinent deduced from palaeomagnetic results is evaluated. A major transition in global tectonics took place during Upper Proterozoic and Lower Palaeozoic times. Older mobile belts developed along reactivated zones with slight movements of cratonic forelands but were related in time to apparent polar movements. The $1,150 \pm 200$ Myr mobile belts formed along a single broad arc characterised by anorthositic and silicic intrusion over a long period of Proterozoic time.

PALAEOMAGNETIC studies of Precambrian and Palaeozoic rocks are yielding at present considerable quantitative information on the distribution and movements of the continental regions, and on their relationship to contemporary orogenic belts during these times. Formation of the supercontinent Pangaea involved the welding of several crustal units in Palaeozoic times¹ and the Caledonian orogenic belt developed along the line of closure of a restricted ocean basin². These are two examples of the operation of plate tectonics during an interval for which seafloor spreading data are not available.

There is now substantial evidence that these examples are amongst the earliest expressions of this phenomenon of plate tectonics. The Upper Proterozoic to Lower Palaeozoic was characterised by a transition in global tectonics from a phase of localised internal deformation of large continental bodies to a phase of fracture, translation and collision of essentially rigid plates³. Palaeomagnetic results have demonstrated that Lower Palaeozoic and older mobile belts in Africa formed between essentially contiguous cratons⁴. A suggestion that Africa and North America have closely similar apparent polar wander (APW) paths before 1,000 Myr (refs 4 and 5) has been substantiated by detailed assessment of the data⁶, and the paths can be closely superimposed over their whole lengths from 1,000 Myr to 2,200 Myr and possibly 2,700 Myr. When this is done the Afro-Arabian and North American regions form a continuous continental body (Fig. 1). Palaeomagnetic results from Gondwanic continents other than Africa, although sparse, are consistent with them occupying their Pangean configuration with respect to Africa in Precambrian times⁶. Australia, however, seems to have changed its orientation sometime between 1,800 Myr and 750 Myr ago. This conclusion has been confirmed by a revised interpretation of the Late Precambrian–Lower Palaeozoic data⁷. Orientations of the Baltic–Ukrainian and Siberian Shields with respect to this reconstruction are uncertain but Donaldson *et al.*⁸ have recognised comparable trends in the North American and Baltic Shield APW curves and have superimposed them to place the two regions close to their present positions between 1,950 and 1,200 Myr.

Present interpretations of Precambrian palaeomagnetic results have two important implications. First they imply that orogenesis, and the behaviour of continental tectonism cannot be directly related to subduction at plate margins. Second, they show that the bulk of continental crust was lumped together as a single body through much of Precambrian time, although paucity of data from European and Siberian regions does not clarify whether or not they were continuously part of such a supercontinent. Here, I examine other evidence for these contentions and examine their implications.

Genesis of mobile belts

The finding that the major Precambrian mobile belts did not form by subduction at destructive plate margins prompts a search for alternative mechanisms. Granites are an important component of these belts and their upward migration through the crust as mobile bodies^{9,10} could cause orogenic folding with zero crustal shortening¹¹. This rise of relatively less dense material through a more dense sedimentary–volcanic cover is a mechanism amenable to

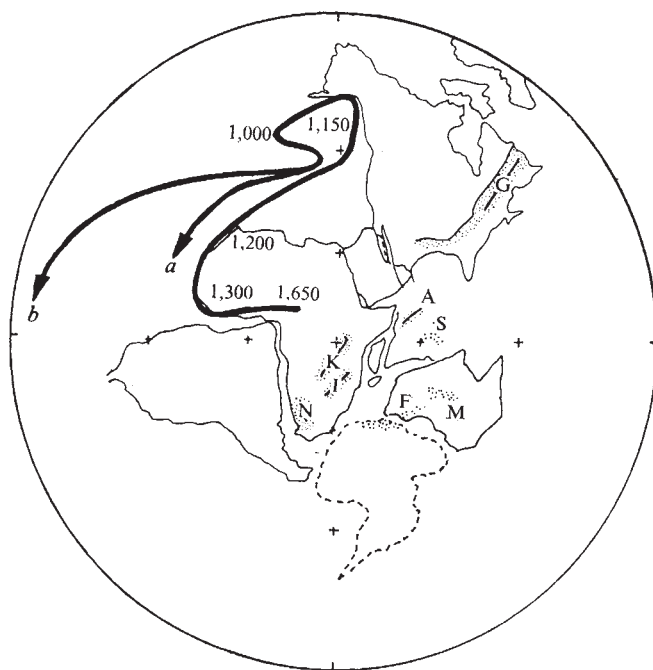


Fig. 1 Stereographic reconstruction of the Gondwana continents and North America before about 1,000 Myr BP derived from palaeomagnetic data⁶. Orogenic belts belonging to the $1,150 \pm 200$ Myr episodes: N, Namaqualand; I, Irumide; K, Kibaran; A, Aravalli; S, Satpura; F, Frazer; M, Musgrave; G, Grenville. The apparent polar wander path spanning their formation is shown: a, North America; b, Africa. Ages in Myr.



Fig. 2 Distribution (stereographic projection) of Precambrian anorthosites (●, refs 37, 39) and granulites (stippled)^{24,42} within the Proterozoic supercontinent. The granulites in part represent relict rocks which have not been reconstituted by later events¹⁹ and may thus indicate a paucity of fluids rather than the absence of any later thermal events.

quantitative analysis, and Ramberg¹² has shown it to be a physically realistic process in mobile belts with a time span in the range 10–100 Myr. Shackleton¹³ has reached a comparable conclusion by regarding the granite magmas as, in part, a differentiate from rising mantle currents, and has argued¹⁴ that progressive addition of granite magmas to the crust occurred during Precambrian time.

This is an obvious mechanism but it is important to clarify the conditions which confine granite intrusion to the narrow zones of basement reactivation along which the Proterozoic belts formed^{3,13,15}. The absence of high pressure–low temperature metamorphic assemblages, and the widespread occurrence of granulites in Precambrian terrains have been rationalised by assuming a higher thermal gradient. Two implications follow from this. First, the crust may have been thinner and second, partial melting must have taken place at shallow depths, presumably leaving a granulite residuum. The migration of fluids from the surrounding rocks may be necessary if significant upward movement of granites is to occur¹⁶ and the crustal levels which they finally reach will then either reflect the point at which the magma chilled beyond its solidus, or the point at which further upward movement was prevented because sufficient fluid could not be abstracted^{16,17}. The concept of fluids is important: base metal mineralisation is practically confined to the Proterozoic mobile belts in Africa, with the cratons barren except for the gold–silver associations of the greenstone belts¹⁸. Paucity of fluids may also explain why large areas of Archaean granulites were not amphibolised in later times¹⁹. It is unlikely that Proterozoic belts have experienced much differential erosion with respect to the Archaean cratons, because platform sediments on the cratons can be correlated with those in the mobile belts²⁰ and their depositional history suggests that the cratons underwent intermittent uplift during Proterozoic times²¹. A possible explanation is that the cratons were added to by underplating with andesitic or granitic material¹⁴ whereas the appropriate fluids concentrated to permit upward migration of granite diapirs only in zones later defined by the orogenic belts and metallogenic provinces.

Given that mobile zones define accessions of heat and/or fluids at the base of the lithosphere, their distribution

must be related in some way to properties of the Proterozoic asthenosphere. The distribution of mobile belts and related features on the Proterozoic continental reconstruction is consistent with such a conclusion.

Precambrian continental distribution

The Proterozoic continental reconstruction (Fig. 1) is applicable from at least 2,200 Myr to shortly after 1,000 Myr BP. It is not possible to match the segments of the APW curves for North America and the Gondwana continents⁶ between approximately 950 and 650 Myr ago. Australia seems to have changed its orientation during Proterozoic times^{22–24} to its Phanerozoic configuration^{25,26}. Before 1,000 Myr sialic crust was distributed as a broad girdle across about 140°, or 15,500 km, of arc and with the inferred placing of the Baltic Shield⁸ this is extended to about 190° or 21,000 km. The agreement of palaeomagnetic results with a single APW curve across this supercontinent⁶ demonstrates that it was disposed on a globe of comparable radius to the present. The reconstruction is based on palaeomagnetic data, with uncertainties which accommodate the limited intercontinental movements necessary to explain the intrusion and deformation of basic dyke swarms, the infolding of sediments and the small relative motions of forelands of mobile belts. Indeed, it is entirely compatible with internal deformation and metamorphism taking place within a coherent entity, as proposed by Sutton and Watson³. Whether or not this is to be regarded as a crustal plate¹¹ in the modern context is academic.

The belts of the $1,150 \pm 200$ Myr mobility, now isolated by later drift, were an essentially unified feature lying on a broad arc close to a great circle spanning the Proterozoic supercontinent. These belts had a protracted history. In Africa the Kibaran belt experienced major tectonism about 1,300 Myr BP with post orogenic granites intruded approximately 1,250 Myr ago and with the subparallel Irumide belt undergoing synorogenic events about 1,100 Myr ago. The

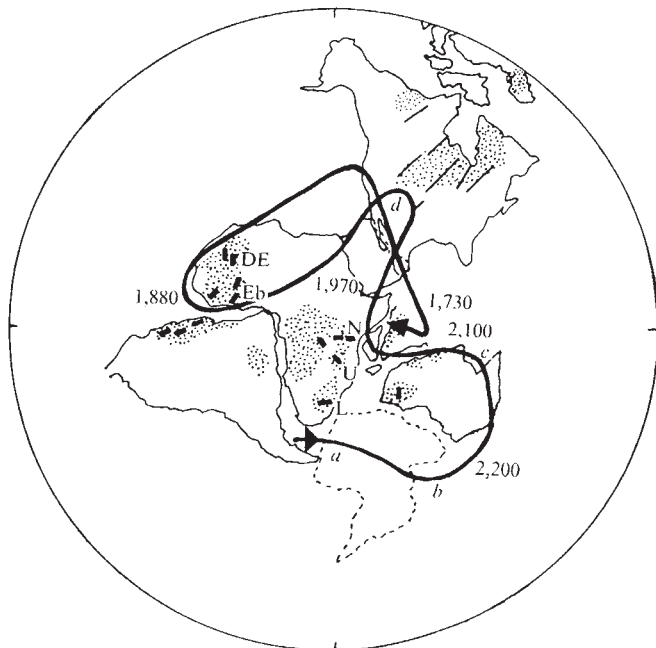


Fig. 3 The relationship of recognisable pre-1,150 ± 200 Myr orogenic belts with cratonic regions^{24,40}, and the apparent polar wander paths executed during their formation. The belts are: L, Limpopo; N, Nyazian; U, Ubendian; Eb, Eburnean; DE, Dorsal Eglabs. The ages of the belts are not precisely defined but the youngest (DE) was folded about 2,100 Myr ago. The straight lines in North America and Greenland are straight belts of late Archaean–early Proterozoic age¹⁹. The generalised APW curve is a composite one which reconciles differences between published APW curves for North America^{8,44} by incorporating a 2,000–1,700 Myr loop identified from African data⁴. a–d, See text; dotted areas, crust unaffected by thermal/tectonic events after about 2,000 Myr BP.

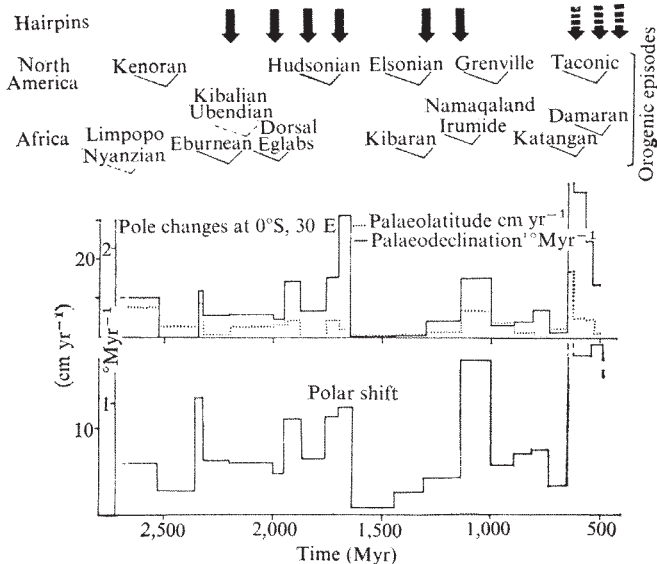


Fig. 4 North American and African orogenic episodes shown in relation to hairpin bends (dashed arrows, the Gondwanic continents) and the rate of apparent polar wandering. The lower histogram shows rates of angular change in the palaeomagnetic pole and the palaeolatitude of a point in central Africa at 30°E, 0°S. Where both of these are large it is probable that the APW reflects crustal drift.

Namaqualand (Orange River) belt of south-western Africa, which experienced a widespread thermal event, with remobilisation of a granite gneiss basement at about the same time, seems to be a linear continuation of these belts, although it has been isolated by the development of the pan-African belts (750–450 Myr). The 1,400–1,050 Myr Frazer–Musgrave belt of Australia²⁷, which, before Phanerozoic drift, connected with rocks of comparable age in the Davis Sea region of Antarctica²⁴, is brought into parallelism with belts of a similar age elsewhere using the Carey–Hurley Rand reconstruction. The Musgrave is regarded by Rutland²⁸ as a narrow zone of basement reactivation with a high geothermal gradient, and it underwent a main phase of metamorphism 1,330 Myr ago.

The continuity of the 1,150 ± 200 Myr orogenic belts is emphasised by identification of rocks about 1,000 Myr old within the pan-African domain of north-eastern Africa (ref. 29 and A. M. Chater personal communication), by the presence of 1,100 Myr old anorogenic rocks in the Arabian Shield³⁰, and by the parallelism of the contemporary Aravalli belt of north-western India (ref. 21 and Fig. 1). Stockwell³¹ and Hurley and Rand²⁴ have distinguished a wide zone affected by a thermal (?) episode about 1,300 Myr ago in the North American Shield, bordering the Grenville Belt, and extensive anorogenic igneous activity of similar age³² has been recognised in southern Greenland, north of the extrapolated continuation of the Grenville belt. In common with the later pan-African episode, restricted zones of linear orogenesis were apparently bordered by wider zones of reheating and igneous intrusion.

If the reconstruction of Donaldson *et al.*⁴ is correct then the 1,150 ± 200 Myr mobile belt may have been even longer than indicated in Fig. 1. Those authors place the Baltic Shield with the Gothide Belt on a great circle continuation of the Grenville Belt. Evidence for a sialic connection is, however, lacking. There is substantial palaeomagnetic evidence^{33,34} that the Grenville Province became attached to the remainder of the North American Shield by predominantly dextral strike-slip plate motion about 1,150 Myr ago and there has been a suggestion⁸ that a similar motion may apply to the juncture of the Gothide Belt with the Baltic Shield. Alternative interpretations are possible, however, and palaeomagnetic data⁴ are not consistent with comparable

movement along the contemporaneous African belts. Along this whole belt there is close spatial relationship between the orogenic belts, postorogenic intrusion and subsequent sedimentation. During and immediately before tectonism the belts were subject to extensive graben faulting and deposition of clastic rocks. Keweenaw sediments formed in tensional rifts along the Grenville Front³⁵ as did Gardar sediments in Greenland and Jotnian sediments in Scandinavia³⁶. Extensive and lithologically similar Upper Proterozoic sequences which outcrop across central Africa include the Bukoban and Kundelungu Groups, developed in a comparable setting to the Kibaran, Irumide and Namaqualand belts^{20,21,29}.

Unlike the later supercontinent Pangaea, which was essentially a transient feature¹ evolved and dismembered by plate tectonic mechanisms, the Proterozoic supercontinent (Fig. 1) existed for a very long period of time (at least 1,200 and possibly 1,700 Myr), and geological events before the 1,150 ± 200 Myr orogenies may be expected to show a simple distribution on the reconstruction. This is indeed the case, and the two broad NE–SW belts of granulites and Archaean cratons which have been recognised on the Pangean reconstruction²⁴ are brought on to a single line (Fig. 2). Also, regions of massive Proterozoic anorthosites, previously regarded as two separate belts ranging from western North America to the Urals and South America to India³⁷, are in fact seen to be one continuous broad belt. These massive anorthosites (Fig. 2) contrast with the anorthosites intimately involved with Archaean granite gneisses³⁸ and their intrusion possibly spanned an interval from 2,200 Myr to 1,000 Myr BP. They represent one facet of magmatism³⁶ that also includes a Rapakivi granite phase, occurring along what is now seen to be a single zone of high thermal activity and block faulting which developed after 2,200–1,750 Myr mobile episodes.

The massive anorthosites are widely regarded as having an upper mantle origin, and it is believed that they required both high temperatures and pressures for their formation³⁹. According to Buddington³⁹ they were intruded during an

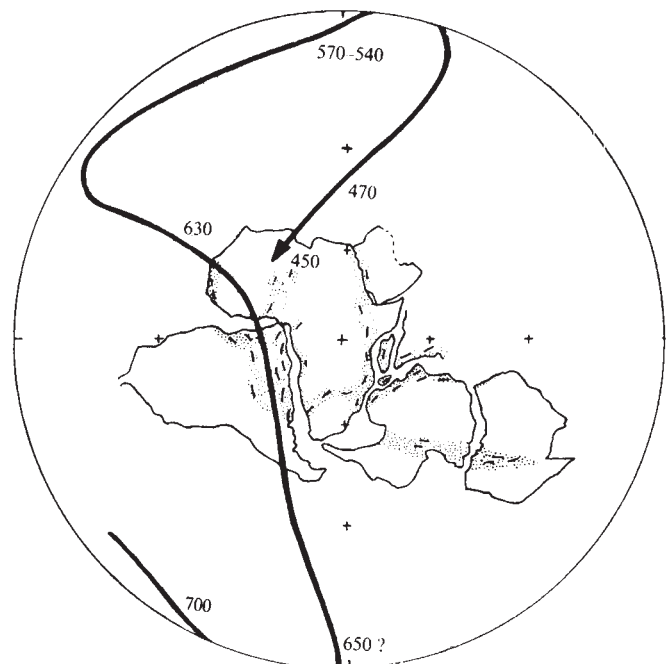


Fig. 5 The pan-African belts (750–450 Myr), showing generalised structural trends and the APW path executed during the interval of their formation. Based on the interpretation of McElhinny *et al.*⁸. The Gondwanaland reconstruction is based on refs 25 and 26; a more southerly position for Madagascar as proposed by Tarling⁴³ would place all continental units to the right of East Africa approximately 10° further south. For simplicity present continental outlines are used in these figures although they include crust accreted in later times.

interval of high temperature gradients such as have not been attained in later times. It is notable that their intrusion ceased at the same time (about 1,000 Myr) as large differential movements of discrete plates first occurred.

The broad belt of magmatism and later tectonic activity is not clearly recognisable as a crustal lineament earlier than about 1,700 Myr, and older Proterozoic belts (>1,750 Myr) have a distribution apparently unrelated to it. The older belts cannot be followed as linear features for distances of more than 100 km. Although that could be a consequence of overprinting by later episodes, where the belt are widely exposed they lie along small circles (Fig. 3 and refs 13, 20 and 40), in contrast to the arc close to a great circle of the $1,150 \pm 200$ Myr belts. Correlation of Proterozoic mobile belts with lines of upwelling¹³ or downgoing¹⁰ mantle convection currents could explain this in terms of a change in the convective systems.

Mobile belts and apparent polar wandering

The parallelism of the Kibaran–Grenville trend and the large contemporary polar shift is a striking feature of Fig. 1. The relationship implies that the continental body executed a large lateral movement along an arc close to a great circle during the interval of deformation and metamorphism. That this and a later apparent polar shift (Fig. 5) represent real translation of the continent, rather than pure polar wandering, is suggested by considerable palaeoclimatic evidence^{6,7}. But it is not possible to equate directly rates of crustal drift and polar wandering, because these will only be equal when the Euler pole is equidistant from the source and palaeomagnetic pole¹. A minimum estimate of crustal drift can be obtained, however, from the change in palaeomagnetic latitude and the amount of local rotation inferred from changes in declination. I have computed these two parameters for a point 30°E , 0°S in central Africa to show that high crustal rates of drift coincide with both the $1,150 \pm 200$ Myr and the pan-African episodes, with low drift rates characterising the quiescent period between the >1,750 Myr and $1,150 \pm 200$ Myr episodes (Fig. 4). The large lateral shifts are terminated by 'hairpins' in the APW curve, which seem to correlate closely with tectonic episodes. This relationship is particularly clear for the $1,150 \pm 200$ Myr events. In these cases the 1,300–1,200 Myr hairpin corresponds to the climax of the Kibaran–Elsonian episode, and the 1,150 Myr hairpin corresponds to tectonic events in the Namaqualand, Irumide and Grenville Belts. In addition, two principal events of the later pan-African episode, the Katangan (680–580 Myr) and Damaran (550–500 Myr) (ref. 38) are both broadly coincident with hairpins (Fig. 5) and the major structural trends of this episode in eastern and western Africa. They could be correlated with the major polar shifts which occurred between 650 and 500 Myr ago although this has yet to be clarified.

Are hairpins and large polar shifts related to earlier orogenic episodes? In most cases age control does not provide adequate information to permit any decision about which part of the APW curve corresponds to particular episodes. There is one possible exception: in the southern part of the West African craton, and in comparable parts of the Guyana craton, the Eburnean tectonism took place along ENE lines about 2,200 Myr ago. Pre- and syntectonic palaeomagnetic poles from the region lie on segment *a–b* of the APW curve (Fig. 3) whereas post-tectonic poles lie on section *b–c* with hairpin *b* occurring synchronously with the tectonic event. Further north in West Africa, in the Dorsal Eglabs, north–south tectonic trends at least 1,970 Myr old are sub-parallel to a segment of the curve defined by syn- and post-tectonic poles from the region (Fig. 3, *c* and *d*). These correlations go a long way towards establishing a relationship between Proterozoic linear

mobile belts and mantle processes. Given that they are produced by zones of asthenosphere which are hotter, and/or contain more volatiles, than surrounding regions, migration of the lithosphere with respect to a mantle source, as evidenced by the polar shifts, would be capable of producing linear belts. Such mantle sources may be comparable to the thermal highs to which recent magmatism is widely related (for example ref. 4).

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Model for the regulation of mRNA translation applied to haemoglobin synthesis

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A kinetic analysis of protein synthesis on ribosomes predicts that certain treatments which reduce the overall rate of polypeptide chain initiation will inhibit translation preferentially of mRNAs with lower rate constants for polypeptide chain initiation. The effects of several inhibitors on α and β -globin synthesis by reticulocyte extracts agree with these predictions.

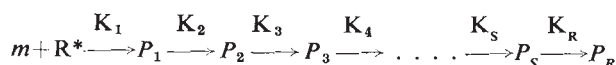
SEVERAL factors determine the rate of initiation of translation of an mRNA molecule. First, different mRNAs seem to differ in the rate of attachment to ribosomes and/or in other stages of initiation of protein synthesis. For example, in the reticulocyte each molecule of α -globin mRNA initiates protein synthesis only 60% as frequently as does each β -globin mRNA^{1,2}. Similarly, the frequencies of initiation of translation of the ten monogenic reovirus mRNAs differ at least tenfold³ and the rates of initiation of translation of the three cistrons of bacteriophage RNA differ more than twentyfold⁴⁻⁸. In this latter case, it has been proposed that the primary nucleotide sequence about the initiation codon and/or the tertiary structure of the mRNA near this codon determine the rate of ribosome attachment^{5,7}.

Second, under certain conditions there are alterations in the overall rate of polypeptide chain initiation in a cell. For instance, during mitosis the rate of chain initiation is only one third of that during other phases of the cell cycle, although the rate of polypeptide chain elongation is unaltered⁹. In reticulocytes, the absence of haemin results in formation of an inhibitor of binding of the initiator Met-tRNA_i to 40S ribosomes¹⁰⁻¹²; initiation of synthesis of all reticulocyte proteins is restricted¹³⁻¹⁵.

In this communication I derive a simple kinetic rate equation for initiation and elongation of polypeptide chains in eukaryotic cells. A principal result of these calculations is that any reduction in the rates of polypeptide chain initiation steps at or before binding of mRNA will result in preferential inhibition of translation of mRNAs with lower initiation rate constants (the poorer mRNAs). I present several new results on translation of α and β -globin mRNA in the rabbit reticulocyte which are predicted by, and consistent with, this conclusion. I will show, further, that changes in the levels or rate-limiting components required for initiation of synthesis of all proteins (for example, initiation factors, Met-tRNA_i, 40S subunits, and so on) could explain many results previously ascribed to mRNA specific factors.

The model

Polypeptide synthesis on ribosomes can be described by the following scheme:



The binding of Met-tRNA_i to the 40S subunit is the first step in protein synthesis in eukaryotic cells¹⁶⁻¹⁹; R^* is the concentration of the Met-tRNA_i-40S ribosome complex able to bind an mRNA; m is the concentration of the mRNA, and K_1 is the rate constant for ribosome binding and formation of the 80S initiation complex P_1 . Although there are several

factors and several steps involved in this process, the use of an overall rate constant K_1 , possibly different for different mRNAs, is sufficient and does not affect the nature of the final rate equation. It is assumed that mRNA-specific factors are not involved in mRNA binding. The polypeptide contains S amino acids and the message S codons; P_i ($i=1, 2, \dots, S$) represents a nascent chain of i amino acids. P_R is the released polypeptide chain. K_i ($i=2, 3, \dots, S$) is the rate constant for addition of the i th amino acid, and K_R is the rate constant for release of the completed polypeptide chain. Ribosomes are known to cover many more codons on a message than those being decoded at any instant; the value L is the number of codons covered by one ribosome.

MacDonald and Gibbs^{20,21} have derived rate equations for polypeptide synthesis in a very general context, and my derivation follows closely their analysis. It is necessary to make several simplifying assumptions in order to yield a relatively simple analytical expression which can readily be compared with experimental data. First, we must assume that rate constant for addition of any amino acid is the same, that is K_i ($i=2 \dots S$) = K_e the single elongation rate constant. Following ref. 20 I define n_i as the probability that an mRNA contains a ribosome with a nascent chain i amino acids long. I make the crucial assumption here that under all conditions ribosomes are uniformly distributed along an mRNA (see below), that is that $n_i = n$ for all $i=1, \dots, S$. It is also assumed that $K_R \leq K_e$ that is that release of the completed chain does not limit the overall rate of protein synthesis. Given these assumptions, the flux of ribosomes, q_e across any given codon in an mRNA is given by equation (10) of ref. 20.

$$q_e = K_e n (1 - nL) [1 - n(L - 1)]^{-1} \quad (1)$$

In the steady state this value represents the net amount of protein synthesised per mRNA, hence the total amount of protein produced per unit time, Q_e , is given by

$$Q_e = m q_e = m K_e n (1 - nL) [1 - n(L - 1)]^{-1} \quad (2)$$

The number of new polypeptide chains initiated per unit time, Q_i is given by the following equation

$$Q_i = m R^* K_1 (1 - nL) \quad (3)$$

where the term in brackets is the probability that the initiation codon is not covered by a ribosome and, hence, available for polypeptide chain initiation. This equation follows from the definitions of K_1 and R^* (ref. 21).

Since, in the steady state, the number of new chains initiated is balanced by the number elongated or completed, we can set $Q_i = Q_e$ and solve for n :

$$n = [L - 1 + K_e (K_1 R^*)^{-1}]^{-1} \quad (4)$$

and combining equations (3) and (4), $Q = Q_i = Q_e$ is given by

$$Q = m R^* K_1 \left[1 - \frac{L}{\frac{K_e}{K_1 R^*} + L - 1} \right] \quad (5)$$

The average number of ribosomes per mRNA is equal to nS ,

and the fraction of codons on a message covered by a ribosome is given by nL . In comparing the translation of two different types of mRNA in the same cell, I assume that K_e (and R^*) are the same for all mRNAs. In the case of greatest interest here, the rabbit reticulocyte, we have shown directly that the rate of elongation of the α and β polypeptides is the same². K_1 (and hence n) will, in general, be different for different mRNAs.

R^* , the concentration of Met-tRNA_f-40S complex capable of binding mRNA, is a key element in this equation; it can be taken as a measure of the overall rate of polypeptide chain initiation in the cell. Reductions in the rate of formation of 40S subunits, or of binding of Met-tRNA_f to them, will result in a lowered value of R^* ; likewise, addition of an inhibitor of mRNA binding, such as poly(I) on aurintricarboxylic acid, will also result in a reduction of R^* .

The crucial assumption in the derivation of equation (5) is that ribosomes are on the average distributed uniformly along an mRNA, that is $n_i = n$ for all i . This is the case for the α and β -globin mRNA in the normal rabbit reticulocyte^{22,23}. In the specific experimental situations considered below—inhibition of polypeptide chain initiation in the reticulocyte—it can safely be assumed that in the steady state ribosomes will continue to be distributed uniformly on the average (but at a lower density). However, in cases where initiation is increased

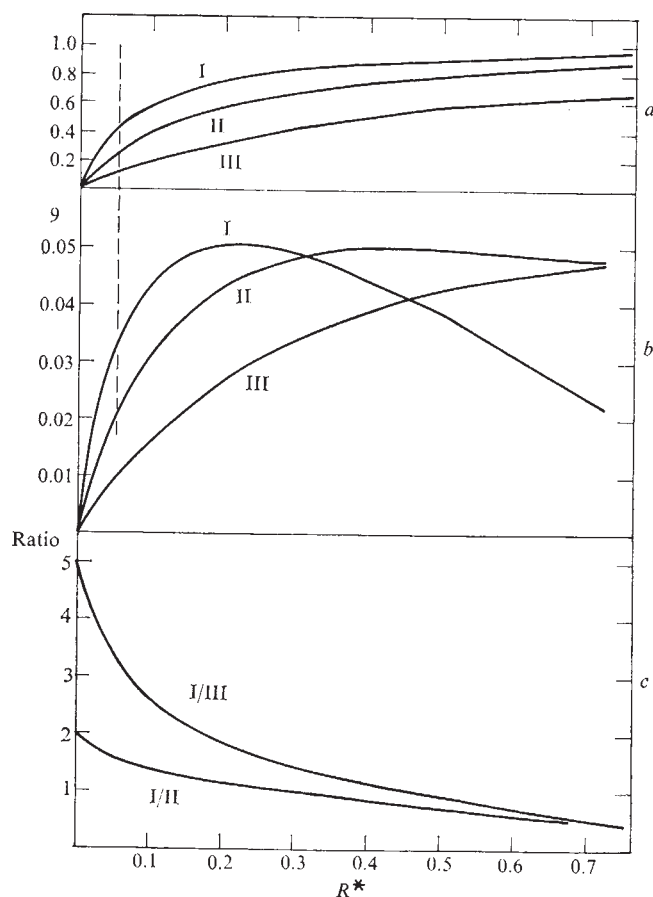


Fig. 1 Theoretical calculation of parameters of protein synthesis as a function of R^* . A value of 1 is assumed for K_e (rate constant for elongation) and 12 is taken for L , the number of codons covered by one ribosome. Rate constants for chain initiation, K_1 , are taken as 1.0 (curve I); 0.48 (curve II) and 0.20 (curve III). *a*, The fraction of codons occupied by a ribosome, nL , plotted as a function of R^* , using equation (4). *b*, Amount of protein synthesised per mRNA, $q = Qm^{-1}$ plotted as a function of R^* , using equation (5). *c*, Ratio of amount of protein synthesised by the different mRNAs, plotted as a function of R^* . As detailed in the text translation of β and α -globin mRNAs can be approximated by curves I and II, respectively. The dashed line represents the conditions obtaining in the normal reticulocyte, where n^β (curve I) = 0.0340 and n^α (curve II) = 0.0211.

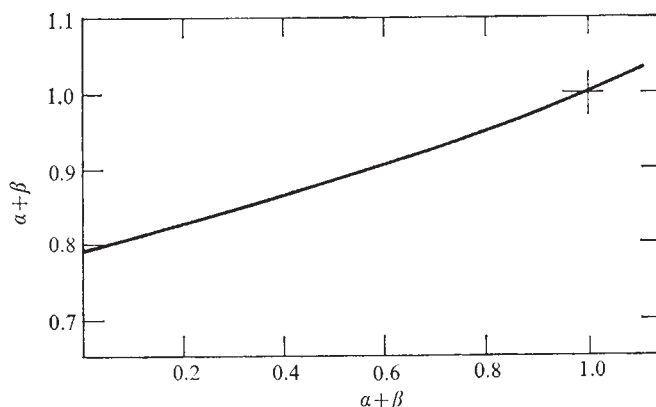


Fig. 2 Theoretical calculation of the effect on relative synthesis of α and β -globin chains by inhibiting polypeptide chain initiation stages at or before binding of mRNA. The values, $K_1^\alpha \div K_1^\beta = 0.48$ and $m^\alpha \div m^\beta = 1.57$ were calculated, as detailed in the text, using only the result that, under normal conditions obtaining in the reticulocyte, each α mRNA contains, on the average, three ribosomes while each β contains five. These values and equation (5) were used to calculate the ratio of α to β chains produced as a function of total globin synthesis; for the latter a value of 1.0 corresponds to synthesis in the uninhibited reticulocyte.

from the normal, relative to chain elongation (high values of R^*) the assumption of uniform distribution may no longer be valid. These cases have been considered theoretically by MacDonald *et al.*^{20,21}.

In Fig. 1*b* the relative translation of three mRNAs is plotted as a function of R^* with values for K_1 set at 1 (curve I), 0.48 (curve II) and 0.2 (curve III). The value of K_e is set arbitrarily at 1, and a value of 12 for L is used. Since a molecule of β -globin mRNA (147 codons) can apparently contain a maximum of 12 ribosomes²⁴ a value of 12 (=144 $\div$ 12) appears reasonable; it is consistent with the size of mRNA protected from nuclease digestion by a ribosome⁸. (The relative positions of the three curves in this panel is unaffected if $L=7$ or $L=20$ is used.)

Note that, with increasing R^* , the rate of protein synthesis increases to a maximum, and then declines. This means that above a certain density of ribosomes on the mRNA ribosomes interfere significantly with movement of each other along the mRNA. (The optimum value of n can be calculated from equation (2) by setting $dq/dn=0$; it is given by equation (7) of ref. 21.) It should be stressed that, because of the assumption $n_i = n$ used in deriving equation (5); the curves in Fig. 1 may not be correct at high values of R^* .

The crucial point to emerge from Fig. 1 is that the relative translation of two mRNAs with different values of K_1 changes monotonically with R^* (Fig. 1*c*). At very low values of R^* the amount of protein synthesised by a species of mRNA is given by

$$Q_{lim} = mK_1R^* \quad (6)$$

Increasing the value of R^* results in proportionally greater stimulation of translation of the mRNA with a lower value of K_1 . Conversely, reducing R^* (inhibiting chain initiation nonspecifically) results in a proportionally greater inhibition of translation of the mRNA with lower K_1 .

Application to synthesis of α and β globin

Cell-free extracts of rabbit reticulocytes synthesise globin for about 1 h at rates comparable to that of the intact cell. These extracts, as do the intact cells, synthesise equal amounts of α and β -globin chains^{1,24}. Each message for a globin contains on the average five ribosomes ($n^\beta = 5/147 = 0.0340$) while each α -globin mRNA contains three ($n^\alpha = 3/142 = 0.0211$)^{1,26}. Since the rate of elongation of the two chains is the same², I conclude that under conditions obtaining in the cell each α mRNA

Table 1 Inhibition of synthesis of α and β globins

Reaction	Time of labelling	% Total globin synthesis	Ratio α : β globins synthesised
Control (plus haemin)	0-10	100	1.02
Minus haemin	0-10	94	0.99
Plus double-stranded RNA	10^{-3} $\mu\text{g ml}^{-1}$ 0-10	92	0.98
	3×10^{-3} $\mu\text{g ml}^{-1}$ 0-10	93	0.96
Plus preincubated lysate	0-10	18	0.83
Control (plus haemin)	10-30	100	1.02
Minus haemin	10-30	17	0.80
Plus double-stranded RNA	10^{-3} $\mu\text{g ml}^{-1}$ 10-30	27	0.86
	3×10^{-3} $\mu\text{g ml}^{-1}$ 10-30	15	0.75
Control (plus haemin)	0-20	100	1.01
Plus ATA 2×10^{-5} M	0-20	45	0.91
Plus ATA 4×10^{-5} M	0-20	21	0.82
Plus poly(dI) $6.6 \mu\text{g ml}^{-1}$	0-20	63	0.92
	$13.2 \mu\text{g ml}^{-1}$ 0-20	17	0.75
Plus poly(dT) $6.6 \mu\text{g ml}^{-1}$	0-20	61	0.89
	$11.4 \mu\text{g ml}^{-1}$ 0-20	38	0.80
Plus pactamycin 5×10^{-8} M	0-20	75	1.11
	1×10^{-7} M 0-20	54	1.13
	2×10^{-7} M 0-20	17	1.29

Reticulocyte cell-free protein synthesis reactions (0.06 ml) have been described in detail^{2,53}. They contained 2×10^{-6} M nonradioactive tyrosine, and ^{14}C -tyrosine ($5 \mu\text{Ci ml}^{-1}$, 480 mCi mmol⁻¹; New England Nuclear Corp.) was added during the indicated labelling period. Unless otherwise noted, reactions also contained $20 \mu\text{g ml}^{-1}$ haemin. Aliquots of the reaction were taken to measure total incorporation of radioactive tyrosine into protein. After incubation, about 600,000 c.p.m. ^3H -tyrosine-labelled rabbit haemoglobin was added. Globin was isolated and digested with trypsin; the tyrosine-containing peptides were resolved by paper electrophoresis and detected by autoradiography (ref. 2). The measure of α -chain synthesis was the average of the ratios of ^{14}C to ^3H radioactivity in peptides αT4 and αT6 ; β -chain synthesis was the ratio of ^{14}C to ^3H radioactivity in peptide βT4 . These three relatively N-terminal peptides were utilised so that peptide labelling represents predominantly (over 98% in the control reaction) polypeptide chains initiated during the course of the cell-free reaction.

initiates protein synthesis only 60% as frequently as does each β mRNA and that there must be 1.7 ($=1 \div 0.6$) times as much α mRNA as β mRNA^{1,2}. Further studies showed that any putative α -mRNA-specific or β -mRNA-specific initiation factors or specific ribosomes do not limit the rate of α or β -globin synthesis (unpublished results of G. Temple and myself).

Since at the value of R^* which obtains in the riboculocyte $n^{\beta} \div n^{\alpha} = 1.6$, I calculate from equation (4) that $K_1^{\alpha} \div K_1^{\beta} = 0.48$. Since $Q^{\alpha} = Q^{\beta}$, from equation (3) I calculate that $m^{\alpha} \div m^{\beta} = 1.57$, in agreement with a previous estimate (ref. 1). The probability that an mRNA will have no ribosomes translating it is given by $(1-n)^s$. For α mRNA in the reticulocyte lysate ($n^{\alpha} = 0.0211$, $s = 142$), this value is 0.049 while for β mRNA ($n^{\beta} = 0.0340$, $s = 147$) it is 0.0062. Recent evidence showed that there is indeed 10 times more α mRNA unattached to ribosomes than β mRNA^{27,28}.

The relative translation of α and β mRNAs, as a function of R^* , are thus given by curves II and I, respectively, of Fig. 1b. In Fig. 2 are plotted the same data but in a form which eliminates R^* — the ratio α : β as a function of the total rate of polypeptide chain initiation (the sum of α and β chains synthesised). Figures 1b and 2 make one crucial prediction: any treatment which (nonspecifically) inhibits the rate of polypeptide chain initiation at stages at or before mRNA binding will preferentially inhibit translation of α -globin mRNA.

Table 1 shows the results of experiments involving six different treatments, all of which do not affect polypeptide chain elongation or termination, and all of which block initiation at steps at or before binding of mRNA. All preferentially inhibit translation of α mRNA. The data in this table follows very closely the theoretical curve of Fig. 2.

When a reticulocyte lysate is incubated in the absence of haemin, protein synthesis is normal for about 8 min (at 30°C). Synthesis continues at a decreasing rate, and ceases by 15–30 min; concomitantly there is buildup of an inhibitor which blocks binding of Met-tRNA_f to the 40S subunit^{10–15}. Table 1 shows that there is equal synthesis of α and β globin during the first 10 min in reactions with or without haemin; and also from 10 to 30 min in the presence of haemin. By contrast, in a lysate incubated in the absence of haemin there is a marked reduction in the ratio α : β synthesised from 10 to 30 min, during the time inhibition is manifest. In a second experiment,

a lysate was incubated for 1 h in the absence of haemin, to build up the initiation inhibitor, and then a small amount was added to a fresh lysate. Again, the amount of α chain made in the second reaction was only 70% that of β (Table 1).

Globin synthesis in the presence of 10^{-2} $\mu\text{g ml}^{-1}$ double-stranded RNA (in the presence of haemin) is similar to that of a reaction in the absence of haemin^{13,29,30}. There is also produced in this case an inhibitor which blocks binding of Met-tRNA_f to the 40S subunit³¹. Table 1 shows that double-stranded RNA has no effect on the amount of α or β chains made during the first 10 min of incubation. By contrast, during the next 20 min, when inhibition of initiation is manifest, there is preferential inhibition of synthesis of α globin.

Aurintricarboxylic acid, at low concentrations, is a specific inhibitor of chain initiation^{32,33}. It blocks the binding of mRNA to the Met-tRNA_f-40S complex (ref. 34 and unpublished data). In kinetic terms ATA can be viewed as lowering the concentration (R^*) of Met-tRNA_f-40S complexes which can bind mRNA. Table 1 also shows that ATA preferentially inhibits initiation of synthesis of α chains.

A large number of synthetic polyribonucleotides and polydeoxyribonucleotides are also specific inhibitors of chain initiation and also block binding of mRNA to the Met-tRNA_f-40S complex (ref. 34 and unpublished data). Like ATA, these can be viewed as reducing the concentration of R^* . We have shown that all 11 polynucleotides tested preferentially block α -chain synthesis³⁵. Table 1 shows recent results for two of those, poly(dT) and poly(dI).

It is interesting that the initiation inhibitor pactamycin which acts at a stage after binding of mRNA to the Met-tRNA_f-40S complex^{36,37} does not preferentially inhibit α -chain synthesis. This result is not in disagreement with the theory, which only pertains to steps at or before mRNA binding. There results give confidence that my theory is correct at least as pertains to synthesis in rabbit reticulocyte lysates.

Application to other systems

That one can alter in a predictable manner the relative translation of different mRNAs by nonspecific changes in polypeptide chain initiation components has implications for other systems. In cases where the rate of cellular protein synthesis is either increasing rapidly with time—such as after fertilisation of the quiescent sea urchin embryo (reviewed in

ref. 38)—or decreasing—such as after infection of cultured cells by poliovirus³⁹—one might reasonably expect that different mRNAs would be affected to different extents.

Although the above calculations assume that mRNA-specific factors do not regulate polypeptide chain initiation, they could easily accommodate them; in this case K_1 for any mRNA would be a function of the concentration of such factors. Whether mRNA specific factors do, in fact, exist is at present controversial. In this context it is useful to point out the three eukaryotic systems available which can translate exogenous mRNA at very high efficiency (greater than 10 molecules of protein produced per mRNA added). The rabbit reticulocyte lysate^{40,41}, the mixed cell-free system of Schrier *et al.*⁴² and the *Xenopus* oocyte (into which the mRNA is microinjected)⁴³. In each of these systems a wide variety of exogenous mRNAs have been translated, and in no case is there any evidence for mRNA specificity. Palmiter has shown for instance, that a molecule of ovalbumin mRNA is translated in a crude reticulocyte lysate with nearly the same rate of chain initiation and elongation as obtains in the intact hen oviduct⁴⁴.

In contrast, RNA-specific factors have been observed in cell-free systems, such as preincubated extracts from mouse ascites cells⁴⁴⁻⁵⁰, in which there is synthesis of, at best, one molecule of protein per molecule of mRNA added⁴⁹. It is clear that in such extracts some component or components required for polypeptide chain initiation (and elongation) are limiting (ref. 51 and reviewed in ref. 52). The present theory shows that addition to such extracts of a component required for initiation for all mRNAs can stimulate preferentially translation of certain mRNAs. It is at present difficult to prove or disprove the notion that mRNA-specific factors, are, in fact, nonspecific components which are required for translation of all mRNAs, particularly since the K_1 constant of a mRNA could be changed considerably by reagents used in its isolation, such as organic solvents and detergents. Addition of unfractionated rabbit globin mRNA to an extract of ascites cells generally results in synthesis of about twice as much β globin as α globin. Nudel *et al.*⁴⁴ reported a factor preparation from reticulocyte ribosomes which will preferentially stimulate translation of α mRNA in the ascites extract. At least in the intact reticulocyte α mRNA has a lower K_1 than β mRNA; the present theory predicts that addition of whatever initiation factor limits synthesis in the ascites extracts would preferentially stimulate α -globin synthesis. Whether this factor, and the others⁴⁵⁻⁴⁹ supposedly specific for translation of β -globin mRNA or myosin mRNA are real entities, artefacts of cell-free synthesis, or, as postulated here, nonspecific components required for all chain initiation, remains for future work.

Finally, the theory developed here is applicable to transcription of DNA by RNA polymerase; under conditions of limitation of some factor required for transcription initiation, the theory predicts a selective inhibition of transcription of those operons with a lower rate constant for polymerase attachment and RNA synthesis initiation.

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Gene deletion as the cause of α thalassaemia

Two independent groups show that the absence of all or part of the globin α -chain gene is the origin of the homozygous α thalassaemia.

The severe form of α thalassaemia is caused by a haemoglobin gene deletion

THE thalassaemias are a group of common genetic disorders characterised by a reduced rate of synthesis of either the α or β chains of human adult haemoglobin (Hb A, $\alpha_2\beta_2$), resulting in associated clinical disorders^{1,2,3}. α Thalassaemia results from a deficiency of α chains, which causes an apparent excess in production of γ chains of foetal haemoglobin (Hb F, $\alpha_2\gamma_2$) in foetal life and an excess of β chains of haemoglobin A in adult life. The excess γ and β chains produce the abnormal tetramer haemoglobins Bart's (γ_4) and H (β_4) respectively. Although the genetics of α thalassaemia are still not fully understood, three important clinical varieties of α thalassaemia result from the interactions of three different mutations in allelic genes: α thalassaemia 1 (α -thal 1), α thalassaemia 2 (α -thal 2), and haemoglobin Constant Spring (CS)^{1,4,5}. The homozygous state for the α -thal 1 mutation (Hb Bart's hydrops fetalis syndrome) results in intrauterine or early neonatal death with a clinical picture of hydrops fetalis and a haemoglobin pattern consisting entirely of Hbs Bart's, H, and Portland ($\zeta_2\gamma_2$), with complete absence of α -globin chain synthesis⁶. The doubly heterozygous states for either α -thal 1 and α -thal 2 mutations, or α -thal 1 and CS mutations, result in Hb-H disease, a moderately severe haemolytic anaemia consistent with survival into adult life¹.

There is evidence that in some populations (including those in south-east Asia), the human α -chain locus is duplicated and there are at least two α -chain loci per chromosome¹. The Hb Bart's hydrops syndrome might result from inactivity of all the α loci whereas in Hb-H disease might be loss of function at one out of the four⁷⁻¹⁴. A deletion of all or part of the sequence of the α -globin genes could prevent the synthesis of functional mRNA. Alternatively, the α -thalassaemia mutations might affect the control of transcription or translation in such a way as to result in a complete inactivity of α -chain gene. The site of mutation could then be remote from the α -globin structural genes.

We have tried to distinguish these different models by a study of the hybridisation of the DNA of an α -thal 1 homozygote to purified probes prepared from globin messenger RNAs with viral reverse transcriptase. We conclude that the α -thal 1 mutation is a gene deletion affecting at least a substantial proportion of all of the α -globin cistrons.

Human globin messenger RNA was prepared^{15,16} from adult reticulocyte-rich blood from a non-thalassaemic haemolytic anaemia (mRNA _{α,β}), umbilical-cord blood from newborn infants obtained at exchange transfusion (mRNA _{α,γ,β}), normal foetal liver (mRNA _{α,γ,β}), and foetal liver from an infant homozygous for α -thal 1. The infant died 20 min after delivery; haemoglobin electrophoresis showed only Hb Bart's and the minor components typical of the Hb Bart's hydrops syndrome⁶.

Both normal and thalassaemic foetal livers were highly erythropoietic. The mRNAs were usually isolated by affinity chromatography using poly(U)—sepharose^{17,18} from total polysomal RNA prepared under conditions minimising RNA breakdown¹⁹. Wherever possible the translational capacity of the mRNAs was tested in a heterologous protein synthesis system, either by microinjection into *Xenopus* oocytes²⁰ or using a cell-free system from Krebs ascites tumour cells²¹ or wheat germ²². The samples were also examined by polyacrylamide gel electrophoresis where possible to evaluate the purity of the mRNAs, but sometimes the small amounts obtained precluded such an analysis.

A complementary DNA copy (cDNA) was prepared to various human globin mRNAs with avian myeloblastosis virus reverse transcriptase²³ using labelled ³H-dCTP only. cDNA was centrifuged on an alkaline sucrose gradient and the material larger than approximately 5S (300 nucleotides) was pooled and used for further hybridisation. cDNAs were prepared using mRNA from adult (cDNA _{α,β}) or newborn (cDNA _{α,γ,β}) reticulocytes as templates; in these cases, unlike that of foetal liver, kinetic analysis demonstrates that only very low amounts of cDNAs are homologous to mRNAs for proteins other than globin²⁴. The newborn reticulocytes synthesise approximately equal amounts of haemoglobins A and F.

Hybridisations of cDNA _{α,β} and cDNA _{α,γ,β} to their template mRNAs show that each cDNA preparation contains approximately 5–10% labelled material which behaved as double stranded even in the absence of hybridisation, and a further 10–15% which did not hybridise to its template even at very high mRNA/cDNA ratios, and which may consist of very short oligonucleotides. None of the data presented here have been normalised (the hybridisation values have not been corrected to allow for the apparently double-stranded or the apparently non-hybridisable cDNA and to equate the observed hybridisation to 100%) since this would obscure the extent of errors which might arise in the experiments. Both cDNA _{α,β} (from adult reticulocyte mRNA) and cDNA _{α,γ,β} (from newborn exchange-transfusion reticulocyte mRNA) hybridised back to their templates to the extent expected²⁴.

The same result was obtained when cDNA _{α,β} or cDNA _{α,γ,β} are hybridised to total mRNA prepared from normal human foetal liver (Fig. 1). But when hybridisation was attempted to total mRNA isolated from liver homozygous for α -thal 1 homozygote, the result was different, with each cDNA hybridising at normal mRNA/cDNA ratios only to approximately 55% of that possible. Since the complexity of these cDNAs is approximately 800 nucleotides (data not shown, see ref. 24), indicating that most of the cDNA is complementary to only two to three mRNAs of the size of globin mRNA, we can interpret the hybridisation observed as due to only β (or β and γ) cDNA components. Any major contamination with cDNAs derived from other mRNAs would have been detected in the kinetic analysis of the hybridisation of cDNA to templates. We conclude that the cDNA _{α} did not hybridise due to the absence of mRNA for α globin from the α -thal 1 liver. This is consistent with the complete absence of α -globin chain synthesis in the thalassaemic foetal material⁶.

Nuclear DNA was prepared from normal human spleen and from liver and spleen of the Hb Bart's hydrops infant. The DNA was purified on hydroxylapatite and sonicated in alkali to an average molecular weight of 100,000 (ref. 25).

The sonicated normal and thalassaemic human DNA were then mixed individually with globin cDNA _{α,γ,β} and allowed to reanneal (Fig. 2). The reannealing of the total nuclear DNA was followed optically by measuring directly the proportion of the A_{260} bound (reannealed) and unbound (single stranded) to hydroxylapatite. Under our conditions about 20% reanneals rapidly, indicating its presence in many copies. This value is slightly lower than previously published²⁶, a discrepancy explicable by the differences in size of DNA fragments and method of hybrid analysis. The remainder of the reannealing curve represents sequences present in one or only a few copies²⁷.

The hybridisation of the human foetal globin cDNA _{α,γ,β} to excess normal human DNA, as measured by binding to hydroxylapatite, demonstrates that the genes for α , γ and β globin all reanneal with the kinetics of the non-repeated portion of the genome. The hybridisation to normal DNA gives a $C_0t_{1/2}$ of approximately 700, slightly more rapid than the $C_0t_{1/2}$ of the total DNA which is 1,000. This might be predicted from

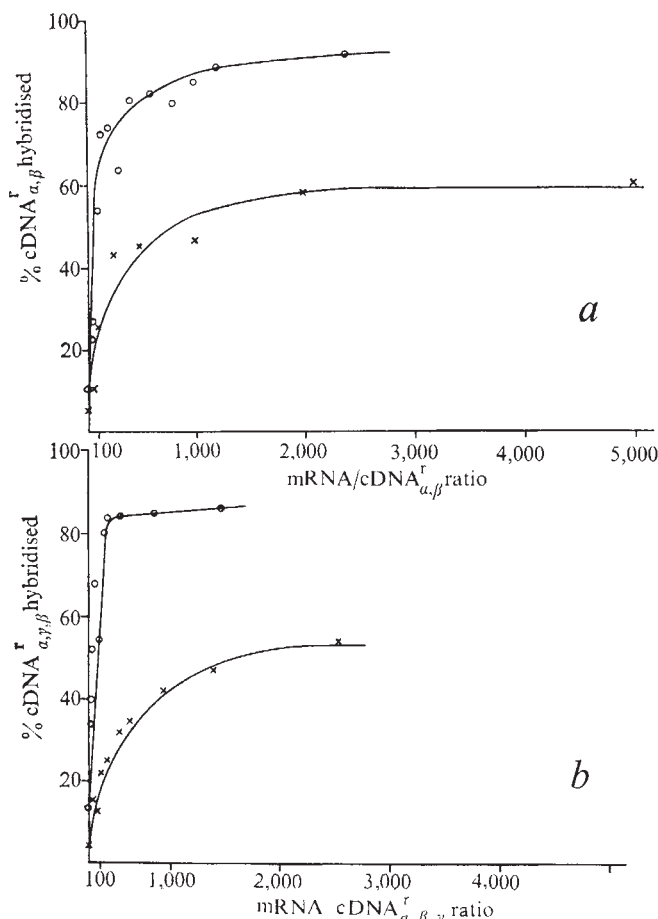


Fig. 1 Hybridisation of liver mRNA from normal and α thalassaemic fetuses to $cDNA_{\alpha,\beta}^r$ and $cDNA_{\alpha,\gamma,\beta}^r$. Human globin mRNA was prepared from ribosome pellets of reticulocyte lysates from adult non-thalassaemic anaemic patients ($mRNA_{\alpha,\beta}^r$) and from newborn infants exchange transfused due to Rh incompatibility ($mRNA_{\alpha,\gamma,\beta}^r$). The pellets were extracted by a phenol-chloroform method³³, and the RNA precipitated by the addition of two volumes of ethanol. The precipitate was dissolved in NETS buffer (100 mM NaCl, 1 mM EDTA, 10 mM Tris, pH 7.4, 0.2% SLS) and poly(A) containing mRNA purified by a cycle of poly(U)-sepharose chromatography¹⁸. Each mRNA was tested in the wheat germ cell-free system²² for biological activity; mRNA from adult reticulocytes directed the synthesis of 57% α and 43% β -globin chain, mRNA from foetal reticulocytes of 55% α , 32% γ and 13% β -globin chain. Counts under globin peaks accounted for at least 80% of the total radioactivity incorporated over the background endogenous synthesis. A single-stranded DNA copy (cDNA) of each mRNA was prepared as described previously^{23,30}, using AMV reverse transcriptase, and with 3H -dCTP (13.3 Ci mmol⁻¹ Amersham) as the only labelled deoxynucleotide; upon centrifugation in alkaline sucrose gradients, both cDNAs, prepared with adult or foetal mRNA had a peak molecular weight of approximately 1.1×10^6 ; material larger than 0.9×10^6 was pooled and used for subsequent experiments. Thalassaemic foetal liver was obtained from an infant with the Hb Bart's hydrops fetalis syndrome immediately after death. Normal foetal liver was obtained from post-mortem material within minutes of death. Livers were homogenised in 3–5 volumes of a solution containing 50 mM NaCl, 1.5 mM MgCl₂, 100 mM Tris, pH 7.6, 100 mM sucrose, 0.1% diethylpyrocabonate, and nuclei were removed by centrifuging twice at 2,000g for 10 min. The supernatant was diluted with 5–10 volumes of NETS buffer, and directly extracted with phenol-chloroform. RNA was ethanol precipitated and mRNA was isolated by poly(U)-sepharose chromatography. 120 pg (specific activity 7.5×10^6 c.p.m. μg^{-1} cDNA calculated assuming dCTP to be 25% of the incorporated deoxynucleotide) of each cDNA were incubated in sealed sterile silicone-treated capillaries with increasing amounts of their template RNAs in 4 μl of a hybridisation solution containing 0.5 M NaCl, 25 mM Hepes, 10 mM EDTA pH 6.8, 50% formamide, 500 μg ml⁻¹ *Escherichia coli* RNA²³. After 6 d at 43°C, the percentage of cDNA which had hybridised was determined by assay with the single-strand specific nuclease S₁ followed by perchloric acid precipitation of the undigested material. The indicated RNA/cDNA ratio has been determined from the amount of added

the fact that there are at least two genes for γ globin and probably two genes for α globin¹. Seventy-five per cent of the cDNA hybridises at a DNA:cDNA ratio of 2×10^7 . The hybridisation of $cDNA_{\alpha,\gamma,\beta}^r$ to the thalassaemic DNA gives a $C_0t_{1/2}$ of approximately 400 but the final amount of cDNA hybridising is only 60%: a deficit of approximately one-quarter compared with normal DNA.

This non-hybridisation of a portion of the $cDNA_{\alpha,\gamma,\beta}^r$ to the thalassaemia DNA suggests that the genes for α -globin have been deleted. To confirm this cDNA enriched in sequences complementary to α -globin mRNA was prepared by two methods. In the first, it was assumed that the foetal liver mRNA from the Hb Bart's hydrops fetalis infant contains no $mRNA_{\alpha}$. $cDNA_{\alpha,\beta}^r$ from adult reticulocytes was hybridised to the mRNA prepared from liver of the Hb Bart's hydrops fetalis infant at an mRNA/cDNA ratio sufficient to give 50% hybridisation of the cDNA. The hybridised sequences (putative $cDNA_{\beta}^r$) were then bound to hydroxylapatite and the non-hybridised $cDNA_{\alpha}^r$ (α -enriched cDNA) which did not bind was collected. At the usual RNA/cDNA ratios the $cDNA_{\alpha}$ hybridises to α thal 1 liver mRNA to a small extent, approximately 20%, as compared to $mRNA_{\alpha,\beta}^r$ or normal foetal liver mRNA, for which hybridisation is to 70%. At very high ratios of approximately 3,000:1 mRNA/cDNA somewhat greater hybridisation (to approximately 30%) occurs between the $cDNA_{\alpha}$ and the mRNA from the α -thal 1 homozygote. We estimate the α -enriched cDNA contains approximately 75% $cDNA_{\alpha}^r$.

The second method for preparing α -enriched cDNA used mRNA prepared from reticulocytes from a patient with homozygous β -thalassaemia as a template for reverse transcriptase. From the ratio of cell-free $\alpha:\beta+\gamma$ chain synthesis the cDNA was estimated to be 80–90% $cDNA_{\alpha}^r$.

The reannealing experiments at vast DNA excess with normal and α thalassaemic DNA were repeated using $cDNA_{\alpha}^r$ as a probe (Fig. 3). The two $cDNA_{\alpha}^r$ probes gave indistinguishable annealing profiles with normal and thalassaemic DNAs. The total DNA reanneals with a $C_0t_{1/2}$ of approximately 1,000 as in the previous experiments and the $cDNA_{\alpha}^r$ reanneals with the normal human DNA to give a curve very similar to that for $cDNA_{\alpha,\gamma,\beta}^r$ with a $C_0t_{1/2}$ of 700. The cDNA hybridises to 70%. In the case of the homozygous α -thal 1 DNA, a quite different picture is seen. Hybridisation occurs only very slowly and a final value of 40% $cDNA_{\alpha}$ hybridised is obtained at a C_0t of greater than 3×10^4 , thirty times greater than the $C_0t_{1/2}$ of non-reiterated DNA sequences, as estimated from the ratios of the C_0t s of the low hybridisation values measured.

The results obtained with normal globin $cDNA_{\alpha,\gamma,\beta}^r$ and $cDNA_{\alpha,\beta}^r$ demonstrate that, as in the case of duck^{28,29} and mouse³⁰, human globin genes reanneal with the non-reiterated component of the DNA, or slightly faster, and are present as only single or a few copies. This is consistent with the current genetic evidence indicating 2–4 human γ and α globin loci¹.

The very different rate of hybridisation for the normal and α -thalassaemic DNAs with both purified $cDNA_{\alpha}^r$ and $cDNA_{\alpha,\gamma,\beta}^r$ cannot be explained by a single nucleotide mutation which affects transcription or translation. The extent of hybridisation of the thalassaemic DNA never exceeds 30% of that of the normal DNA, even at very high C_0t s, demonstrating the deletion of all, or at least a substantial part, of the genes for α -globin mRNA. Since there are almost certainly two α -chain genes in this population, this suggests that they are closely linked. The $cDNA_{\alpha}$ hybridisation to 70% with normal total DNA demonstrates that α -globin genes are present in large excess relative to cDNA in these experiments.

9S RNA (as calculated by polyacrylamide gel electrophoresis analysis of poly (U)-bound RNA). The material which bound to the poly(U)-sepharose column (poly(A)-containing RNA) was hybridised to $cDNA_{\alpha,\beta}^r$ (a) and $cDNA_{\alpha,\gamma,\beta}^r$ (b). The unbound material did not contain any appreciable amount of globin sequences as determined by hybridisation to cDNA. The indicated amount of RNA refers to the total poly (U)-bound material. Hybridisation of a, $cDNA_{\alpha,\beta}^r$ and b, $cDNA_{\alpha,\gamma,\beta}^r$, to normal (○) and α -thal 1 (×) foetal liver mRNA.

A deletion of more than half of the α -globin gene at all loci would give the annealing profiles seen in Figs 2 and 3. As the cDNA is transcribed from the poly(A) sequence on the 3'-end of the mRNA, it is particularly rich in sequences at the 3'-end of the gene, coding for C-terminal sequences of the protein, and therefore the extent of the deletion will be underestimated. Moreover, the purified cDNA _{α} ^r is still contaminated with approximately 25% sequences complementary to other globin mRNAs.

A component of the reannealing seen with the purified cDNA _{α} ^r and α -thal DNA might be due to mismatched hybridisation to the genes for the α -type chain zeta (ζ). This early embryonic chain forms tetramers with γ chain in haemoglobin Portland ($\zeta_2\gamma_2$) in early embryonic life, and is known to be present in α -thal 1 homozygotes at approximately 1–5% of the total globin⁶. Preliminary studies indicate that it contains some α -chain-like sequences, particularly at the C-terminal end³². Furthermore, it behaves functionally as an α chain, since the tetramer $\zeta_2\epsilon_2$ (the probable formula for haemoglobin Gower 1³³) shows complete cooperative function. The slow rise in hybridisation between cDNA _{α} ^r and mRNA from α -thal foetal liver at very high mRNA/cDNA ratios may also be due to mismatched mRNA–cDNA α hybrid formation.

We conclude, therefore, that a substantial, and in all probability complete, deletion of the genes for α globin is the molecular mechanism which produces the α -thal 1 disorder and hence, in its homozygous state, the Hb Bart's hydrops syndrome. Extrapolating from our results with α -thal 1, it seems likely that in many cases α -thal 2 results from the loss of a single α -gene locus. Since there is now strong evidence that the heterozygous state for Hb CS also mimics the clinical picture of α -thal 2, it is possible that some cases of α -thal 2 result from a molecular mechanism other than a deletion.

From a practical point of view, it is perhaps discouraging that a disorder such as this severe form of α thalassaemia, which causes a major public health problem in many parts of the

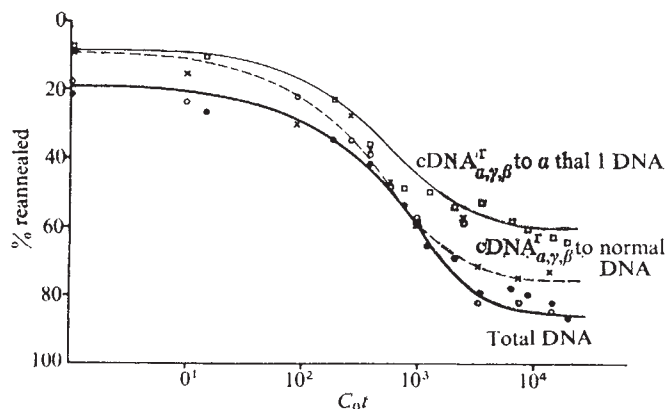


Fig. 2 Reassociation of cDNA _{α,γ,β} ^r to excess normal or α -thalassaemic total DNA. DNA was prepared from nuclei of normal human spleen or of hydrops foetalis liver with hydroxylapatite²⁵, and sonicated in 0.1 N NaOH to an average size of approximately 300 nucleotides. DNA fragments were neutralised, ethanol precipitated, redissolved in distilled water and dialysed three times against 20 volumes of water. cDNA _{α,γ,β} ^r (0.5 ng) was mixed with excess (20×10^6 l⁻¹) normal or α -thalassaemic DNA at a concentration of 8.3 mg DNA ml⁻¹ in 0.12 M sodium phosphate, pH 6.8. 120 μ l aliquots of the solution were sealed in sterile silicone-treated capillaries; the mixtures were denatured for 10 min at 100°C, then annealed at 60°C. At the appropriate times, samples were removed and fractionated on hydroxylapatite columns³⁰. Reannealing of total DNA was followed by measuring spectrophotometrically the proportion of DNA eluted as single-stranded material (at 0.16 M sodium phosphate, pH 6.8) and double-stranded material (at 0.4 M sodium phosphate). Radioactivity of the 0.16 M and 0.4 M phosphate fractions was determined by counting directly in Instagel (Packard). In all cases, at least 95% of the radioactivity, or of the total DNA, was recovered as single-stranded or reannealed DNA by hydroxylapatite. The percentage of radioactivity in single-stranded DNA is shown by $\times$ (normal DNA) and $\square$ (α -thalassaemic DNA); that of absorbance in single-stranded DNA by $\circ$ (normal DNA) and (α -thalassaemic DNA).

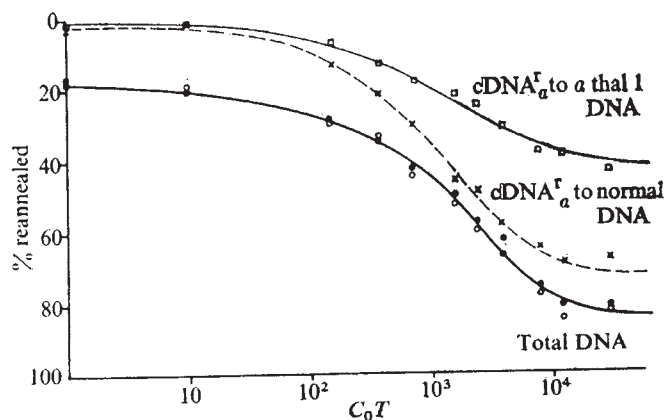


Fig. 3 Reassociation of α -enriched cDNA to excess normal or α -thalassaemic total DNA. α -Enriched cDNA (0.4 ng) prepared by hydroxylapatite adsorption was mixed with excess (25×10^6 l⁻¹) normal or α -thalassaemic DNA at a concentration of 5 mg ml⁻¹ in phosphate buffer, and hybridised as in Fig. 2. Percentage of radioactivity in single-stranded DNA: $\times$ (normal DNA) and $\square$ (α -thalassaemic DNA). Percentage of absorbance in single-stranded DNA: $\circ$ (normal DNA) and $\bullet$ (α -thalassaemic DNA). In a control experiment α -enriched cDNA was hybridised with a 20×10^6 excess of *E. coli* DNA. 3% of the cDNA _{α} was eluted with the double-stranded fraction at C_0t 0, 8% at C_0t 4,500, 9% at C_0t 10,000. The slight increase of cDNA eluted in the 0.4 M phosphate fraction at very high C_0t after hybridisation with *E. coli* DNA has been previously reported³³.

world, results from a gene deletion, since this precludes any possibility of correction at the post-transcriptional level.

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Genetic lesion in homozygous α thalassaemia (hydrops fetalis)

HOMOZYGOUS α thalassaemia (hydrops fetalis) is invariably fatal to afflicted infants within 30–40 weeks *in utero* or soon after birth¹. In this form of α thalassaemia both the α chain of haemoglobin and the α -globin mRNA are undetectable^{2,3}. To define the nature of this defect we have examined the DNA from two cases of hydrops to determine whether major alterations in the structural gene for α globin are present.

For these studies we prepared complementary DNA (cDNA) to total globin mRNA ($\alpha + \beta$) from a non-thalassaemic patient with autoimmune haemolytic anaemia, as well as cDNAs specific for either α or β -globin mRNA sequences. The procedures are detailed in the legend to Fig. 1. Evidence for the specificity of the cDNAs was obtained by measuring the rates of annealing (expressed as $C_{\text{r}}t$ (mol s⁻¹ l⁻¹)) to an excess of globin mRNA. The two cDNAs annealed at the same rate to globin mRNA from a non-thalassaemic individual (Fig. 1a). When globin mRNA from an individual with haemoglobin H disease was used, the presumptive α -globin cDNA annealed at a rate about ten times slower than that of the β -globin cDNA (Fig. 1b). In this disease there is a reduction of α -globin chain relative to production of β -globin chain and a corresponding reduction in the α -globin mRNA^{9,10}. When an aliquot of the mRNA used in the above experiment was translated with the cell-free system from the Krebs II mouse ascites tumour⁹, the ratio of α to β globin synthesised was 1:9. Thus the different rates of annealing observed in Fig. 1b are consistent with the presumption that the cDNAs used are specific for α and β -globin sequences.

We then used these ³²P-labelled cDNAs to detect α and β -globin nucleotide sequences in DNA extracted from the livers of two Chinese infants with homozygous α thalas-

saemia and, as a control, a stillborn infant without thalassaemia. Annealing of the ³²P-labelled cDNA during the reassociation of the unlabelled cellular DNA was monitored by hydroxylapatite fractionation. A large excess of cell DNA was used to ensure that even sequences represented a single time in the human genome would anneal with the cDNA. Our results are summarised in Fig. 2, with experimental details provided in the legend. In each experiment we in-

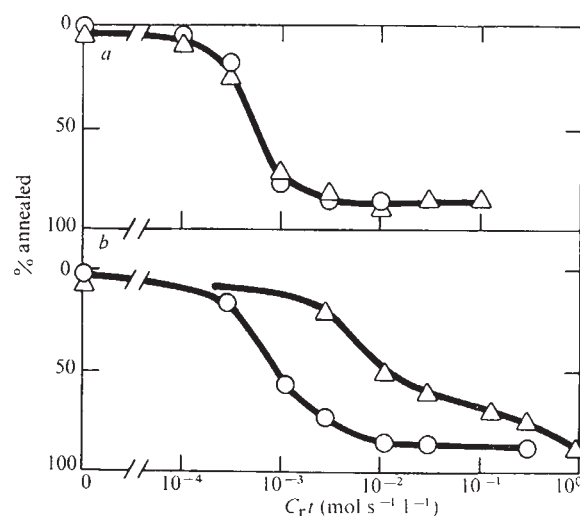


Fig. 1 Annealing of globin cDNA to globin mRNA. Reticulocytes were obtained from non-thalassaemic individuals or from patients with haemoglobin H disease. RNA was extracted with phenol and sodium dodecyl sulphate⁴, and analysed by rate zonal sedimentation. RNA migrating in the range 7–11S was pooled and used without further purification. These RNAs were translated using the cell-free system from Krebs II mouse ascites tumour⁹ and the product was analysed by chromatography on CM-cellulose⁶. The product translated from non-thalassaemic RNA contained equal amounts of α and β globin and from haemoglobin H RNA the ratio of α to β globin synthesised was 1:9. Both RNAs were transcribed into cDNA using RNA-directed DNA polymerase purified from Prague C strain of Rous sarcoma virus. The conditions of synthesis were as follows: 0.1 U ml⁻¹ polymerase⁷, 0.4 μ g ml⁻¹ RNA template, 0.1 μ g ml⁻¹ oligo (dT)_{12–18} (Collaborative Research), 100 μ g ml⁻¹ actinomycin (Calbiochem), 0.1 M Tris (pH 8.1), 0.008 M MgCl₂, 0.02 M β -mercaptoethanol, 80 μ M dATP, 80 μ M TTP, 80 μ M dGTP, 0.8 μ M (α -³²P) dCTP (115 Ci mmol⁻¹, New England Nuclear Corp.). Synthesis was for 2 h at 37° C using a reaction volume of 3 ml. After the addition of 50 μ g of yeast RNA, the nucleic acids were extracted with sodium dodecyl sulphate and pronase, followed by a single phenol extraction at room temperature. The cDNA was precipitated with ethanol and treated with alkali (0.6N NaOH, 37° C; 1 h) to remove RNA. The solution was neutralised and again precipitated with ethanol. The cDNA was then annealed to an excess of its template (normal or haemoglobin H mRNA) under the following conditions: 0.3 M NaCl, 0.001 M EDTA, 0.02 M Tris (pH 7.4), at 68° C. That cDNA which annealed to RNA was isolated by batch elution from hydroxylapatite⁸ and then passed through a column of Sephadex G-50 (40 $\times$ 0.9 cm). The excluded volume was collected, treated with alkali as described above and reprecipitated with ethanol. That cDNA transcribed from RNA of the individual with haemoglobin H disease was used as a probe for β -globin sequences without further purification. The cDNA transcribed from the normal RNA was subjected to further fractionation in an attempt to obtain probe specific for α -globin sequences. First, the unfractionated cDNA was annealed to a limited extent with haemoglobin H RNA. After incubation, that cDNA which had not annealed (30%) was isolated by hydroxylapatite fractionation and Sephadex G-50 chromatography, and subsequently treated with alkali and precipitated with ethanol as described above. This adsorption process was repeated three more times in an attempt to remove sequences complementary to β -globin mRNA. The remaining unadsorbed cDNA was finally annealed to an excess of normal RNA. The annealed cDNA was recovered. The experiment depicted in the figure shows evidence that this cDNA is specific for α -globin sequences. The presumptive α -globin (Δ) and β -globin ($\circ$) probes were examined for their ability to anneal to an excess of RNA either from a normal individual (a), or from a patient with haemoglobin H disease (b). Hybridisation was assayed by batch elution from hydroxylapatite and results are plotted against the product of RNA concentration and time ($C_{\text{r}}t$).

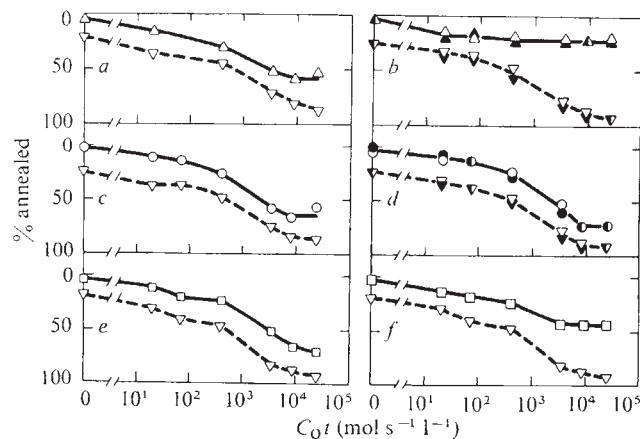


Fig. 2 Annealing of labelled globin cDNA to cellular DNA. Nucleic acid was extracted from the livers of two patients with hydrops fetalis using sodium dodecyl sulphate and pronase followed by two room temperature phenol extractions. After treatment with ribonuclease and further phenol extractions, the DNA was reduced in size to about 2×10^6 daltons either by shearing at 50,000 pounds per square inch¹² or limited depurination¹³ (0.3N NaOH, 100°C for 20 min). HeLa cells in suspension culture were labelled with ^3H -thymidine for 48 h and the DNA was isolated as described above. Single-stranded ^{32}P -cDNA was prepared as described for Fig. 1, and annealed under the following conditions: 5 mg ml⁻¹ cell DNA, 2400 c.p.m. ml⁻¹ ^{32}P -globin cDNA, 24,000 c.p.m. ml⁻¹ ^3H -HeLa DNA, 0.5 M NaCl, 0.002 M EDTA, 0.04 M Tris (pH 7.4), at 68°C. Incubations were performed in 80 μl reaction volumes, under mineral oil, for from 20 min to 120 h. Annealing was assayed using hydroxylapatite¹⁴ and plotted against C_0t values corrected to standard conditions¹⁵. *a* and *b*, α -globin cDNA (Δ , $\blacktriangle$) was annealed either with normal or hydrops DNA respectively. Similarly (*c*) and (*d*) refer to β -globin cDNA ($\circ$, $\bullet$) annealed with normal or hydrops DNA. Unfractionated globin cDNA ($\square$) transcribed from normal mRNA was also annealed to normal (*e*) and hydrops DNA (*f*). In each panel of the figure the ^3H -labelled DNA (∇ , $\blacktriangledown$) acts as an internal control on the observed rate of annealing. *b* and *d*, The DNA from two cases of hydrops was tested separately and the results are shown by the open and closed symbols.

cluded a small amount of ^3H -labelled human DNA from HeLa cells and its annealing was followed to determine the rate of reassociation of the cellular DNA. Figs 2*a* and *c* show that α and β -globin cDNAs annealed at about the same rate to DNA from the non-thalassaemic foetus. In contrast, α -globin cDNA did not anneal significantly to DNA from the hydrops foetuses (Fig. 2*b*) although β -globin cDNA reacted normally (Fig. 2*d*). A similar result was obtained when unfractionated globin cDNA ($\alpha + \beta$) was used. The extent of annealing with non-thalassaemic DNA (Fig. 2*e*) was about twice that observed with hydrops DNA (Fig. 2*f*). We interpret that data to mean that at least part of the α -globin gene is absent from the DNA in hydrops; the amount of the β -globin genes appears to be normal.

This conclusion is subject to several qualifications. First, although the cDNA used is specific for α globin, it is not a uniform representation of the structural gene. The average size of the cDNA used in the experiment was only 380 nucleotides, when measured by sedimentation in alkaline sucrose. The complete α -chain mRNA is composed of about 630 nucleotides¹⁶; about 60 of these are contained in the 3'-terminal poly(A) segment¹⁷, adjacent to which is a segment of at least 93 nucleotides which are not normally translated¹⁸. Since the cDNA used in our experiments was presumably transcribed from the 3'-end of the mRNA, it is likely to represent only a part of the sequence of the α -globin structural gene.

Second, the observed rates of annealing of the cDNA to cell DNA depend on the relative excess of sequences in the cell DNA that are complementary to the cDNA. The mass ratio of cell DNA/cDNA used was 6.7×10^7 . If we assume the human haploid genome contains 3×10^9 nucleotide pairs and the average length of the cDNA is

380 nucleotides, then the ratio of unlabelled to labelled sequences is 8.5:1. This also assumes that the α -globin gene is represented only one per haploid genome. Actually there is evidence that the gene might be duplicated¹⁹ so that our sequence excess might be twice the value calculated above. To confirm that these experiments were performed with adequate quantities of cell DNA we have performed annealings with as much as a fivefold greater excess of cell DNA relative to cDNA and have seen no more than a 10% increase in the extent of annealing. We therefore deduce from the annealing rates in Fig. 2*b* compared with those in Fig. 2*d*, that the hydrops DNA contains less than 2% of the α -chain sequences detected in normal DNA. Assuming that the DNA of each somatic cell is identical, then part or all of the α -chain gene is actually deleted. In addition, if the α -chain gene is normally duplicated then in the hydrops both loci must be affected.

In summary, we have shown that for homozygous α thalassaemia in Chinese subjects, the cellular DNA lacks certain sequences normally found in α -globin mRNA.

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letters to nature

Highly polarised radio outburst from Cygnus X-3

WE report here the results of extensive observations of a new series of outbursts from Cyg X-3 which we commenced following the recent IAU telegram alert from Sir Martin Ryle. A high degree of linear polarisation has been discovered in one of the recent outbursts. The degree of polarisation changes over a period of 17 d and reaches a maximum of 14%. The observations indicate a high degree of order in the source magnetic field and that a thermal plasma coexists with the radiating relativistic electrons. In addition, a new upper limit of $0.05''$ has been obtained for the angular size of Cyg X-3, together with an improved position for the source.

In September 1972 radio emission from Cyg X-3 increased by three orders of magnitude in the course of a few days, reaching a maximum of 22 Jy only to decline again during the following week.¹ Since then some 17 outbursts have been recorded by individual observers and by a monitoring program at the Algonquin Radio Observatory². The present observations covering the period May 14 to 31 include measurements of flux density, linear and circular polarisation at two frequencies, 2,695 and 8,085 MHz. The observations were obtained with the NRAO four element phase-stable interferometer at Green Bank. The measured flux densities for Cyg X-3 are shown in Fig. 1 with values of the spectral index $\alpha = \log(S_{8085}/S_{2695})/\log(8085/2695)$, where S_{8085} and S_{2695} are the measured flux densities at the two frequencies. The flux densities are calibrated

in terms of the flux densities of 3C48, 3C147 and 3C286 and have an uncertainty of ≤ 0.1 Jy or 3% whichever is the greater. These three sources were also used as linear polarisation calibrators. Their adopted flux densities and polarisation parameters are given in Table 1. Observations of these and other

Table 1 Adopted values of flux density and polarisation for the primary calibration sources. m is the degree of linear polarisation and χ is the measured position angle of the electric vector

Source	$f=2,695$ MHz			$f=8,085$ MHz		
	S (Jy)	m (%)	χ (degrees)	S (Jy)	m (%)	χ (degrees)
3C48	9.00	2.1	60	3.30	5.5	110
3C147	12.60	0.7	44	4.90	0.7	163
3C286	10.10	9.2	30.9	5.20	11.4	34.4

secondary flux density calibrators were interlaced with Cyg X-3 observations once every hour. A more detailed discussion of the observations and their interpretation will be presented elsewhere.

An attempt was made to detect circular polarisation. We obtained an upper limit of 0.5% at both frequencies. This limit and the theory of synchrotron radiation³ place a corresponding upper limit of about 0.02 gauss for the magnetic field in the radiating region.

The position of Cyg X-3 as measured with the maximum interferometer baseline of 35 km was found to be:

$$\begin{aligned} \alpha &= 20 \text{ h } 30 \text{ min } 37.620 \text{ s} \pm 0.005 \text{ s} \\ \delta &= 40^\circ 47' 12.85'' \pm 0.05'' \end{aligned} \quad \left. \vphantom{\begin{aligned} \alpha &= 20 \text{ h } 30 \text{ min } 37.620 \text{ s} \pm 0.005 \text{ s} \\ \delta &= 40^\circ 47' 12.85'' \pm 0.05'' \end{aligned}} \right\} 1950.0$$

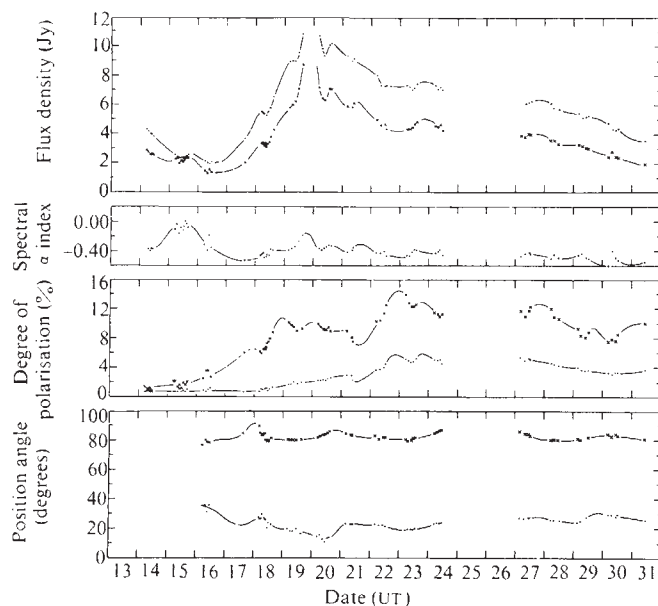


Fig. 1 The flux density, spectral index, degree of linear polarisation and position angle of the radio emission plotted as a function of UT date. For an unresolved source of linear polarisation P is normally expressed in terms of the degree of linear polarisation m and the position angle χ of the polarised component. The measured values of m and χ , corrected for instrumental polarisation, are also shown. From an analysis of the measurement uncertainties and from the constancy of the instrumental polarisation during the entire observing period, we are confident that the trends conveyed by the solid curves are real and not produced by noise or variations in the instrumental polarisation. $\times$, 8,085 GM7; $\bullet$, 2,695 GM7. Observations cover the period May 14 to 31.

This position is consistent with previous measurements⁴⁻⁶ but the quoted errors of the present measurements are significantly lower. A new upper limit on the angular size was obtained from simultaneous observations of Cyg X-3 with the 35 km baseline and shorter baselines over a wide range of hour angles. The mean fringes visibilities measured at 2,695 and 8,085 MHz were 1.00 ± 0.03 and 1.03 ± 0.11 , respectively, and no hour angle dependence was detected. The minimum fringe visibility consistent with the 8,085 MHz observations corresponds to an angular size for a circularly symmetric source of uniform brightness of $\leq 0.05''$. If we adopt for the distance the lower limit of 10 kpc (ref. 7) this corresponds to a linear size of $\leq 8 \times 10^{15}$ cm.

The radio emission observed from May 14 to 31 represents a portion of at least two large outbursts which began about May 11, 1974 (P. C. G., V. A. Hughes, A. Woodsworth and E. R. S., unpublished). We believe that the decay in the emission between May 14 and 16 constitutes the end of the first outburst. The second outburst began about May 17. The spectral index and polarisation of the radio emission together clearly indicate a nonthermal emission process characteristic of synchrotron emission from relativistic electrons. If the electrons have a power law energy spectrum ($N(E) \propto E^\gamma$) the observed mean spectral index ($\alpha = -0.4$) yields $\gamma \approx 1.8$ throughout most of the period of the observations. In addition, the spectral index profile shows a tendency for α to decline from -0.2 to -0.6 during the observations. This general behaviour is characteristic of radiation from an optically thin cloud of relativistic electrons in which there are energy losses which occur preferentially at the higher energies. With regard to the fact that the source is optically thin the second of these outbursts is similar to the third and fourth outbursts observed in 1972 at similar frequencies^{4,8}. The first two outbursts in 1972, however, exhibited an optically thick phase at and below 2,695 MHz.

One of the principal differences between the May 2 outburst and all of the September 1972 outbursts is the slow rise in flux density from May 17 to May 20, followed by an even slower decline in flux density from May 20 to 31. The September activity was characterised in general by a more rapid rise and decline in the flux density. There is, however, evidence in the fine structure of the May activity that the outburst profile may be decomposed into a series of smaller outbursts, with sharp rise times, occurring so frequently that they are unresolved. The picture suggested is that relativistic particles are repeatedly injected into a region which remains essentially optically thin. On two occasions (May 15.5 and May 19.5) the spectrum flattens significantly and we presume that the associated minor outbursts correspond to particle injection in the form of a new region of emission which is either optically thick at 2,695 MHz or possesses a flatter energy spectrum in the radiating particles.

The most readily observed characteristic of the polarisation is a general increase in the degree of polarisation at both (Fig. 1) frequencies, rising from about 1% to 14% at 8,085 MHz between May 14 and May 23 followed by a slight decline. The position angle of the electric vector is remarkably constant at both frequencies, but there are significant deviations, of the order of 10° , from a mean value. The electric vector position angles at the two frequencies differ by about 60° . The latter observation leads to a mean rotation measure of about -100 rad m^{-2} . This value is consistent in magnitude and sign with the galactic Faraday rotation in the Cygnus direction, although the latter quantity is extremely uncertain. On the other hand, the deviations in rotation measure shown in Fig. 2 indicate that there is some Faraday rotation in the source as well.

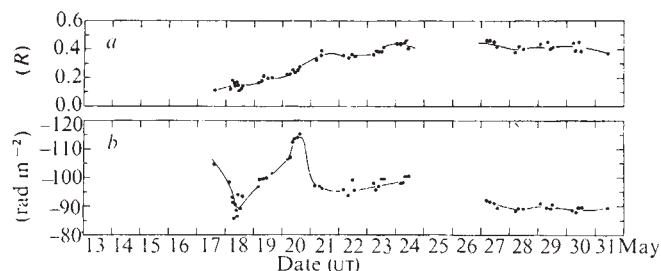


Fig. 2 The derived depolarisation ratio (a) and rotation measure (b) plotted as a function of UT date.

The degree of polarisation is lower at 2,695 MHz than at 8,085 MHz. The 'depolarisation ratio' $R = (\text{degree of polarisation at 2,695 MHz}) / (\text{degree of polarisation at 8,085 MHz})$ varies from about 0.1 on May 17 to about 0.45 on May 24 and remains roughly constant thereafter. The most direct interpretation of this result is that the radiation at 2,695 MHz is partially depolarised by Faraday dispersion produced by an ionised thermal plasma associated with the source. The polarisation observations therefore make it possible to deduce some of the characteristics of this depolarising medium.

Burn⁹ has described the depolarisation behaviour of simple source models with internal and external Faraday dispersion. In particular, he computes the dependence of the observed degree of polarisation and position angle on the amount of depolarisation for varying degrees of order in the magnetic field.

We may have made a qualitative comparison of the Cyg X-3 polarisation behaviour with Burn's model for internal Faraday dispersion in the case of spherical geometry. If the model is applicable the 8,085 MHz radiation is not significantly depolarised by Faraday dispersion but that Faraday effects are partly responsible for depolarisation at 2,695 MHz. The ratio R is then a measure of the amount of depolarisation by Faraday dispersion at 2,695 MHz. So the degree of polarisation at 8,085 MHz is determined entirely by magnetic field disorder, and the position angle is determined by the field orientation. Over the observed range in the depolarisation ratio ($R = 0.1 \rightarrow 0.45$)

the position angle of the electric vector at 2,695 MHz would be expected to vary by about 15° or less depending upon the detailed structure of the magnetic field. This expectation is certainly comparable with the observed slight systematic variation in the position angle at 2,695 MHz. The position angle at 8,085 MHz remains essentially fixed. If this interpretation is correct, the observed increase in the degree of polarisation at 8,085 MHz suggests an increase with time in the degree of order of the magnetic field within the source during the course of the outburst.

The observed increase in R suggests a continuous dilution of the depolarising medium with time. With regard to the internal Faraday dispersion model, this suggests either an expansion of the emitting region and depolarising medium together, or a movement of the emitting region relative to the depolarising medium.

If the ordered component of the field is primarily responsible for the depolarisation effects, then when $R = 0.45$, we have, from equations (18) and (19) of Burn⁹,

$$nH_{\parallel}L \approx 4.7 \times 10^{14} \text{ gauss cm}^{-2}$$

where n is the thermal plasma density, $H_{\parallel}$ is the line of sight component of the uniform magnetic field strength, and L is the size of the emitting region.

If $L \sim 10^{15} \text{ cm}$ and the field strength is about 0.01 gauss, consistent with the limits on the magnetic field derived for the first outburst¹⁰ in September 1972, we would require an electron density on the order of 100 cm^{-3} to explain the depolarisation observed. The optical depth τ of this plasma would be about 10^{-7} at 2,695 MHz if $T = 10^4 \text{ K}$, and would therefore be optically thin at the observed frequencies as required.

In a reanalysis¹⁰ of the September 1972 outbursts have shown that a thermal plasma within the emitting region plays the major role in determining the optical depth in the first two outbursts. The high plasma densities $\sim 10^7 \text{ cm}^{-3}$ calculated for the first two September 1972 outbursts would produce a complete depolarisation of the radio emission by Faraday effects. Indeed, most observers (see ref. 11) found only an upper limit to the degree of polarisation. The one previously reported detection of linear polarisation¹ has since been found to be accountable for by an instrumental effect. The high polarisation observed in May is consistent therefore with the fact that the outburst is optically thin.

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Distances to the sources of observed gamma-ray bursts

We suggest that the brief, intense outbursts of hard X rays, or soft γ rays of cosmic origin which have been termed γ -ray bursts¹ are mainly, though not necessarily entirely, of Galactic origin. More specifically, the γ -ray bursts reported so far² seem to originate in the local (Orion) spiral arm of our Galaxy, at distances of, typically, several hundred parsec. If we assume that the sources radiate into 4π sr, the absolute luminosities are $\sim 10^{39}$ erg per event. (It may be significant that this is of the order of the predicted Eddington limit radiation from accretion disks around collapsed stars.)

Although observations of the phenomenon of γ -ray bursts are well established—the first report¹ has been substantiated by numerous independent observations^{3–7}—the data are by no means extensive. In particular, only a couple of dozen events² have been confirmed by two or more independent detector systems, and less than half of these have provided useful information on source directions. The directions are of critical importance to estimates of source distance, because the more direct methods, such as correlation in time or position with reported cosmic events or stellar objects of known distance, respectively, have so far failed. The amount of data is unlikely to increase significantly in the next few years, so we are obliged to squeeze what we can from existing data.

At present, directional information exists for 11 events. Five of these have alternative positions (see ref. 2), with approximately the same Galactic latitudes (because of the fortuitous orientation of the orbital plane of the satellites), but quite different Galactic longitudes. The latitude distribution can be examined in order to test the isotropy of the source distributions. Figure 1 shows the number of sources above a given latitude as a function of that latitude, for 10 γ -ray

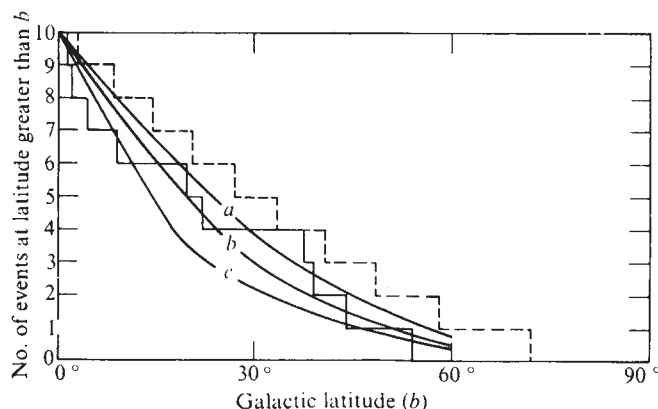


Fig. 1 Actual and isotropic integral latitude distributions compared with calculated distributions for extreme Population I objects. Solid line histogram, integral distribution in Galactic latitude of 10 γ -ray burst sources; dashed line histogram, distribution expected for 10 sources distributed uniformly. Calculated curves for sources of constant intrinsic luminosity; a, detectable out to 200 pc; b, 300 pc; c, 500 pc.

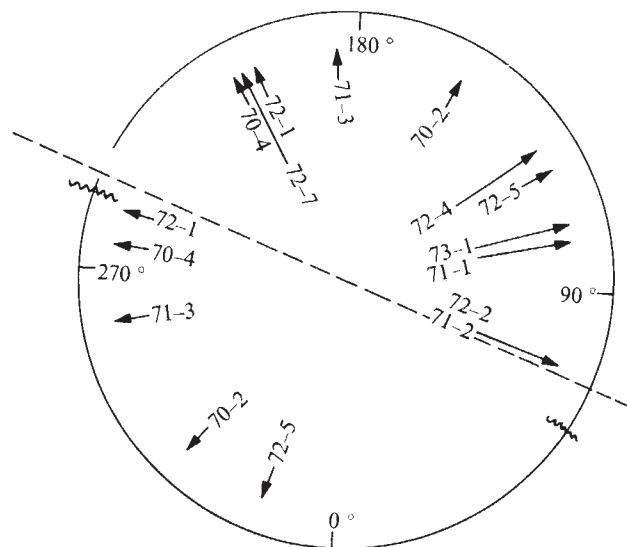


Fig. 2 The distribution in Galactic longitude of 11 γ -burst sources. Long arrows, unique directions; short arrows (which occur in pairs, one on each side of the dashed line), alternative positions for the source; dashed line, direction of local spiral arm. The sources are named according to the system established in ref. 2.

bursts and for 10 sources distributed perfectly isotropically (one at a very low latitude is not included; its addition strengthens an argument discussed later). The real sources seem slightly deficient at high latitudes, a result, unfortunately, of no great statistical weight.

The longitudinal distribution is shown in Fig. 2. Here, there is something statistically more significant. The orientation of the local spiral arm of this Galaxy is indicated and all six of the uniquely determined directions lie on the side away from the Galactic centre. The Sun is known to lie near the inner edge of the spiral arm. If the detected sources lie at distances from the Earth, which are greater than the distance of the Sun from the ill defined inner edge of the arm (a distance of, at most, a few tens of parsec) but less than the distance (around 1 kpc) to the next (Sagittarius) arm in towards the Galactic centre, then they would, if associated with the young Population I stars of the spiral arms, be expected to lie in the longitude range $\sim 70^\circ$ – $\sim 250^\circ$.

Consider a simple model of source distributions in which the sources have a z distribution approximating that of the Galactic gas⁸ and intrinsically faint stars⁸. This distribution is appropriate because it is close to that of the bright O and B supergiants that have been associated with cosmic X-ray sources^{9,10}. Initially, we assume all the sources to have the same absolute luminosity and calculate, from the z distribution given by Weistrop⁸, curves giving the fraction of sources above a given latitude, for any given maximum source distance (Fig. 1). It can be seen that a maximum distance of ~ 300 pc is not a bad fit. Assuming that some of the observed range of flux reflects variations in intensive luminosity, the curve can be made to fit even better, including a proportion of extra luminous sources around longitudes of $\sim 70^\circ$ and $\sim 250^\circ$, at distances of up to ~ 1 kpc.

An argument independent of direction can also be adduced for a Galactic origin. If the sources were distributed uniformly throughout space then the number detectable above a given flux level would follow the well known ' $-3/2$ power law'. If $N(S)$ is the number of sources producing a flux greater than S then $N(S) \propto S^{-3/2}$, where $\alpha = 3/2$. For sources confined to an infinite, thin sheet, $\alpha = 1$, whereas for sources strung out along an infinite line, $\alpha = 1/2$. This argument does not require the sources to have uniform, intrinsic luminosities.

Cline and Desai (unpublished) have examined the IMP-6 data for γ -ray bursts smaller than those detected by the Vela satellites. Because of a number of difficulties, the results were

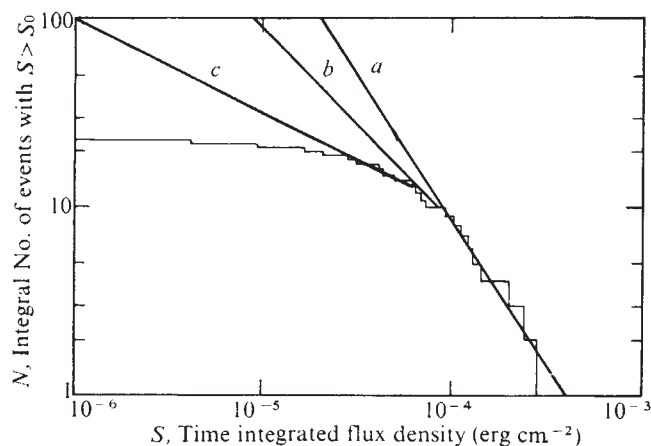


Fig. 3 A plot for 23 γ -ray bursts, detected by the Vela satellites, of the number of sources, $N(S)$, with an energy flux S or greater, against S . Vertical and horizontal scales are logarithmic. *a*, Expected curve for sources distributed uniformly throughout a volume ($-3/2$ law); *b*, source distributed uniformly over a plane (-1 law); *c*, sources distributed uniformly along a line ($-1/2$ law). (All this assumes that the detector is sensitive to infinitely small fluxes, that is, no threshold effects are present.)

not conclusive, but they did report that there seemed to be a deficiency of smaller bursts—less than the number predicted by an $\alpha \approx 3/2$. If $\log N(S)$ is plotted against $\log S$ for the bursts detected by the Vela satellites, the curve of Fig. 3 is obtained. It was at first assumed that threshold effects in the detector trigger logics could explain the break away from the $-3/2$ power law below $\sim 10^{-4}$ erg cm^{-2} , but a more careful examination of the problem now suggests that the deviation is mostly real. If the sources are Galactic the $\log N \sim \log S$ curve (less a multiplicative constant) can be predicted from a simple count of, for example, O-associations or H II regions, which would have a similar distribution. The resultant curve matches well the observed curve of Fig. 3 down to $S \approx 3 \times 10^{-5}$ erg cm^{-2} , where the trigger threshold effects begin to predominate. The curve below $\sim 7 \times 10^{-5}$ erg cm^{-2} actually follows a line with α less than 1, but above 1/2. This is partly a result of the narrowness of the spiral arms and partly because of reduced stellar density further from the Galactic centre.

Two γ -ray bursts, events 71-2 and 72-2 (see ref. 2) each had one of their two possible source directions very close to that of Cygnus X-1 (I. B. Strong, *et al.*, unpublished). If this tentative identification is correct it agrees well with the argument for a Galactic origin. The distance, 2.5 kpc, is about a factor of three greater than may have been expected, but if γ -ray bursts do originate in binary systems of the Cygnus X-1 type (a possible black hole?) it may be that Cyg X-1 itself is a particularly luminous member of its class.

In conclusion, we warn against the automatic assumption that all the γ -ray bursts have similar origins. It may be so, but we are really discussing cosmic X-ray and γ -ray emission processes of brief duration and there may well be more than one such process. Also, although we consider the argument for a galactic origin to be sound, there may be a small proportion of extra galactic events included in the data.

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Gamma radiation from the Crab Nebula above 35 MeV

OBSERVATIONS of electromagnetic radiation from the Crab have been made to at least 1 GeV (ref. 1) and possibly as high as 10^{12} eV (refs 2 and 3). The Crab is unique among strong X-ray sources in that the major component in the low energy range (1-10 keV) shows little or no temporal variation. A small ($\lesssim 10\%$ at 10 keV) pulsed component from NP0532 is observed in the X-ray region, but its strength relative to the constant component increases with increasing X-ray energy. Gamma ray observations of the Crab in the 15-100 MeV range have yielded rather uncertain results with reported upper limits^{4,5} and fluxes⁶⁻¹⁰ in apparent or near conflict. As the reported intensities were just marginally detectable above atmospheric background with the balloon experiments which produced the observations, this seems understandable.

Here we report observations of the Crab above 35 MeV made with the high energy gamma-ray telescope flown on SAS-2. The detector and technique have been described^{11,12}.

The data were obtained during the week of December 14-21, 1972. The Crab direction remained about 5° from the experiment axis for the entire exposure. The absolute

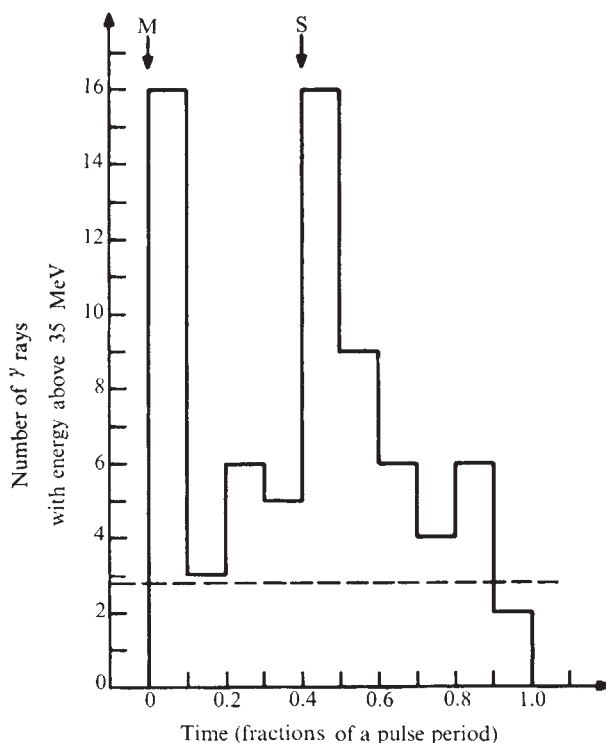


Fig. 1 Distribution of γ -ray arrival time in fractions of a pulse period for γ rays above 35 MeV from the direction of NP0532. M and S refer to the predicted main and secondary pulse times as determined from radio observations (J. M. Rankin, private communication) — — —, background flux.

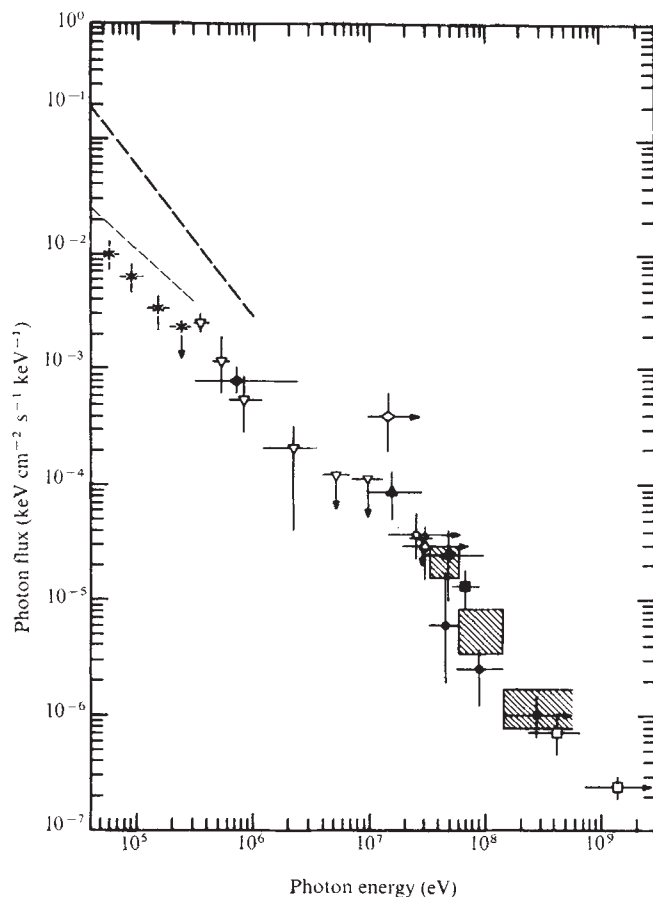


Fig. 2 Spectrum distribution of fluxes observed from the region of the Crab nebula. —, $0.7E^{-0.9}$ pulsed flux¹⁹; ---, $21.8E^{-1.29}$ total X-ray flux¹⁹; hatching, SAS-2 total flux; ●, SAS-2 pulsed flux; △, ref. 17; □, ref. 1; ○, ref. 10; ▲, ref. 7; ■, ref. 6; open diamond, ref. 8; △, ref. 9; solid diamond, ref. 18; ★, ref. 14; vertical arrow, ref. 5.

timing accuracy of the SAS-2 satellite and the ground station was sufficient to allow separation of the pulsed component. Because of the finite angular resolution of the SAS-2 detector, an angular cone of acceptance for γ rays must be included in the final analysis. We used a 6° cone for γ rays with measured energies from 35 to 100 MeV and 4° for energies above 100 MeV. Appropriate account was taken of the portion of the flux which is calculated on the basis of the known detector angular resolution to fall outside these circles.

The arrival time of each γ ray was compared to the radio pulse time using predicted pulse arrival times (J. M. Rankin, personal communication) and derived as described in ref. 13 (Fig. 1). An independent timing analysis was made using a program based on a pulse prediction program (D. C. Backer, personal communication).

Peaks occur at both the positions of the main and secondary radio pulses, and there is a constant component above the general background from the Crab region. Although the statistics are limited, the probability of finding by chance 16 γ rays in a bin where a peak was predicted, when the average based on the eight bins where no peak was expected is five, is less than 10^{-4} . The probability of finding peaks of the observed size in both bins is then extraordinarily small. As the absolute timing uncertainty for SAS-2 was about 1 ms, selecting markedly smaller intervals of time than those shown in Fig. 1 does not produce peaks of increased significance.

The total flux of γ rays above 35 MeV is estimated to be $(15.8 \pm 4.2) \times 10^{-6}$ photons $\text{cm}^{-2} \text{s}^{-1}$, and that of the pulsed component, using bins 1 and 5 only (Fig. 1) is estimated at

$(6.2 \pm 2.8) \times 10^{-6}$ photons $\text{cm}^{-2} \text{s}^{-1}$. The total flux above 100 MeV is $(3.2 \pm 0.9) \times 10^{-6}$ photons $\text{cm}^{-2} \text{s}^{-1}$, and the pulsed component above 100 MeV is $(2.2 \pm 0.7) \times 10^{-6}$ photons $\text{cm}^{-2} \text{s}^{-1}$. These results are shown in Fig. 2, along with data from other experiments. The unpulsed component seems to become a progressively smaller fraction of the total flux as the energy increases. Above 140 MeV, only a 95% confidence upper limit of 0.7×10^{-6} photons $\text{cm}^{-2} \text{s}^{-1}$ can be set for an unpulsed component, whereas a finite flux of $(1.30 \pm 0.46) \times 10^{-6}$ photons $\text{cm}^{-2} \text{s}^{-1}$ was seen for the pulsed component. The situation contrasts markedly with the low energy X-ray region, where only 10% of the radiation is pulsed (ref. 14).

The most striking feature of the spectral distribution of the electromagnetic radiation from the Crab between 10^4 and 10^9 eV, is the shift from a predominantly unpulsed total flux at the lower energy, to one which is predominantly pulsed. The spectral shape of the pulsed emission, although only poorly known, can be approximated by a power law with an energy spectral index of $\alpha \sim 1$ to about a GeV. Qualitatively, this picture is consistent with the Shklovsky¹³ model of synchrotron emission from the speed of light cylinder with a steady flux of much lower energy photons from the region of smaller field. With a magnetic field in the Nebula between 10^{-4} and 10^{-3} gauss electrons must be accelerated up to 10^{12} – 10^{13} eV to produce γ rays in the 100 MeV energy region. Such electron energies have been predicted in models of particle acceleration for the Crab pulsar.

It is interesting to compare the γ -ray results discussed here with those for the Vela region reported by Thompson *et al.*¹⁶. The γ -ray fluxes from the two regions above 100 MeV are nearly equal; the Vela flux is $(5.0 \pm 1.2) \times 10^{-6}$ photons $\text{cm}^{-2} \text{s}^{-1}$. But the Crab spectrum in the γ -ray region seems to be a simple extension of the X-ray spectrum, whereas in the case of the Vela source the γ radiation is quite hard and substantially larger than an extension of the X-ray spectrum to that energy region. The Vela intensity and spectra are consistent with the γ radiation being the result of about 3×10^{50} erg of cosmic-ray nuclei from the Vela supernova interacting with the local matter.

It is not inconsistent with the data, for the Crab supernova also to have released a few times 10^{50} erg of cosmic rays, because as it is about four times further away, the γ radiation from cosmic-ray interactions would be approximately 16 times less intense, assuming the local matter density is not grossly different from 1–2 nuclei cm^{-3} . The γ -ray intensity near 100 MeV from supernova nucleonic cosmic rays then, would be more than an order of magnitude less than the pulsed radiation at the present time.

So, a consistent picture of the present data ascribes a very different origin to the γ -ray intensity from the Crab to that from Vela, with the Crab γ radiation resulting from the same mechanism responsible for the X-ray emission, and the Vela γ radiation resulting from the nucleonic component of the cosmic rays interacting with the local matter.

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Gamma-ray bursts from neutron star glitches

THERE is no lack of proposed explanations for gamma-ray bursts. A large amount of observational facts and theoretical arguments has been assembled recently¹. The basic facts are:

- The occurrence, several times every year, of short bursts of high energy photons. These events last $\sim$ seconds and cover the spectral range between keV up to about 1 MeV.
- The total flux at the earth is roughly between 3×10^{-6} erg cm⁻² and 5×10^{-4} erg cm⁻².
- The bursts consist of distinct components, often two in each event. The time interval between components ranges from about 1 s up to about 30 s (a much more rapid intensity variation exists within each component).
- The angular distribution is roughly consistent with isotropy. The sources are either extragalactic or fairly local (say, within a few hundred parsecs). An analysis of the data now available yields a relationship for the 'number of courses as a function of intensity' which has been interpreted as favouring a local origin².

Here we propose that the cosmic gamma-ray bursts arise from the very abundant population of old, slowly spinning neutron stars which are no longer observable as pulsars. It is supposed that the bursts are emitted because of magnetospheric activity related to glitches.

The number density of defunct pulsars in the solar neighbourhood has been estimated to be around 3×10^{-2} pc⁻³ (ref. 3). An order of magnitude more of these objects would still be compatible with the Oort limit $0.2 M_{\odot}$ pc⁻³. Within 10 pc one can expect 10^2 and possibly 10^3 old neutron stars.

A typical burst of order 10^{-4} erg cm⁻² implies a source power $10^{34} R_{10}^2 \text{ pc}^2 \text{ erg}$ (R_{10} is the distance in parsecs). The observed frequency of several events per year and their intensities are compatible with the number of defunct pulsars if each of them emits, say, 10^{35} to 10^{36} erg every 10 to 100 yr.

This possibility is suggested by recent observations of period

irregularities in old pulsars. R. Manchester and coworkers have monitored half a dozen pulsars during four years and have definite evidence for two irregularities ('glitches'). In the first case (PSR1508 +55) they have found a glitch of relative magnitude $\delta = \Delta\Omega/\Omega \gtrsim 2.2 \times 10^{-10}$ (ref. 4); in the other case (PSR0329 +54) the glitch is of order $\delta \approx 1.8 \times 10^{-10}$ (Manchester, private communication). These observations suggest a rough figure of about one glitch per pulsar every 10 to 20 yr.

A considerable fraction of the energy released in a glitch probably causes a sudden change of the magnetospheric fields and leads to the production of fast particles. How much energy is actually released is, however, model dependent. If a glitch is a sudden change in stellar shape caused by internal relaxation processes the stellar magnetic field (well anchored in the conducting surface) will change by an amount $\Delta B = \delta B$. This will give rise to an induced electric field in the conducting magnetosphere, to Alfvén waves and to particle acceleration. The total energy release is of the order $\delta(B^2/4\pi)(4\pi/3)R^3$. Typical parameters $R = 10$ km $B_0 = 10^{26}$ erg cm⁻³, $\delta = 2 \times 10^{-10}$ correspond to an energy release around 10^{34} erg. Note, however, that this is a lower limit because some surface rearrangements can alter B without changing the stellar moment of inertia.

In alternative models the glitches are caused by magnetospheric instabilities and the energy release can be much larger.

In this type of model the maximum glitch would have a magnitude⁵

$$\delta_{\max} = (\Delta\Omega/\Omega_{\max}) \approx (B_0^2 R/M\Omega^2)$$

A glitch of intensity δ would release an energy of the order

$$(\delta/\delta_{\max}) B_0^2 R^3 \approx \delta M R^2 \Omega^2$$

If $\delta = 2 \times 10^{-10}$, $P = 6$ s, a typical neutron star would release about 4×10^{36} erg.

If the magnetic field is around 3×10^{12} gauss and $\delta = 2 \times 10^{-10}$ the induced electric field is sufficient to impart relativistic energies to the magnetospheric particles. Indeed

$$|E| dt = R/c \Delta B \approx 10 \text{ V cm}^{-1} \text{ s}$$

The energies which can be reached are clearly insufficient to radiate high energy photons through the curvature radiation mechanism. On the other hand, any amount of energy pumped into motions transverse to the field lines will be radiated away. Low energy electrons radiate near the Larmor frequency—field measured in units of 10^{12} gauss). Relativistic electrons they emit photons $kh\nu \approx 10 B_{12} \text{ eV}$ (B_{12} is the perpendicular field measured in units of 10^{12} gauss). These high energy photons, however, would not be able to escape from the star surroundings if their energy ε (in MeV) satisfies the relationship $\varepsilon B_{12} \gtrsim 4$ (they would instead give rise to electron-positron pairs).

If the bursts arise near the neutron star surface and the emission is not highly collimated with the field lines, our model naturally accounts for the spectral range covered by the bursts and predicts a sharp cutoff around 1 MeV.

Pulsars turn off after their periods lengthen to about 3 s, at a typical age $t \approx 10^8$ yr. If the torque remains unchanged, then $P \propto (\text{age})^{1/2}$ and the dead pulsars should have periods in the range from 3 to 30 s (most of them will have the slowest rotation). If, however, the braking and the radiation mechanisms are associated, (for instance if both of them result from the current flow through the polar surface) most dead pulsars would have periods not much greater than that at turnoff.

These considerations suggest a possible interpretation of the time structure of the bursts. If the emission arises in a specific sector of the magnetosphere and the activity lasts longer than one rotation period, then the bursts can be expected to disappear from our view because of rotation and should reappear some time later (say, 1 s up to about 30 s later). If

there were no evolutionary effects in the active region there would be an exact periodicity for the repetition of pulse groups within an individual event. In real life such an explosive phenomenon is likely to be accompanied by spreading and motions of the active region: this would prevent genuine periodicities. Rotation could also introduce other horizon effects and possibly explain why some bursts are detected not during their rise but when they already have peak intensity.

Finally, we note that the neutron star model for bursts can potentially suggest the presence of two characteristic time scales. The first is in the millisecond range and represents the time for an Alfvén wave to cross the magnetosphere. The other is roughly around 10 s, the time needed by an Alfvén wave to cross the star interior.

It is easy to see that the lack of gamma-ray bursts in coincidence with the glitches in the Vela and in the Crab pulsar does not pose a serious problem for our model. Indeed, because of the very large distance of these sources, a gamma-ray burst would have been detected only if the power was enormously greater than in the other cases. It is of obvious interest to monitor the timing of nearby pulsars: this could give hope of detecting a coincidence between glitches and gamma bursts.

To conclude, we note that there is no reason to exclude the possibility of glitches in neutron stars also occurring when they accrete mass from a nearby companion. It is then possible that some X-ray binaries may emit gamma-ray bursts. If Cyg X-1 is confirmed to be a source of bursts (I. Strong, private communication) our model would require the accreting object to be a neutron star rather than a black hole.

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Note added in proof: After this paper was submitted, M.R. received a preprint from A. I. Tsygan (Leningrad) containing similar ideas.

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Pulsed 10–30 MeV gamma rays from PSR0833-45

OF the more than 100 known pulsars the one in Vela, PSR0833-45 is the most like the Crab pulsar in many of its characteristics. It has the second shortest period (89 ms), the second largest dP/dt , undergoes sudden 'glitches' or decreases in its period¹ and is associated with a known supernova remnant². The many optical searches for pulsed radiation have so far been negative³. In the X-ray region a positive result was reported for 0.5 to 1.5 keV but 29 ms earlier in phase than the radio frequency pulse⁴. At 23 to 80 keV evidence was found for pulsed emission with a period 155 ns different from the radio period⁵. Later X-ray observations have not detected any pulsed radiation⁶⁻⁸. Observations from Uhuru show an X-ray source at this

position⁹, but the time resolution was too long to detect pulsing. Grindlay *et al.*¹⁰ have reported a possible indication of pulsed emission above 3×10^{11} eV.

Here we report detection of pulsed emission from PSR0833 at 10–30 MeV. A sharp peak is observed coincident with the radio pulse. There is also some evidence for precursor emission.

The balloon-borne spark chamber detection system is the same as used earlier for the detection of γ rays from NP0532 (ref. 11). On flight 571 from Longreach, Queensland, on November 16, 1972, Vela was observed¹² from 1620 to 2320 UT. An azimuthal orientation system kept the spark chamber axis pointed south at a zenith angle of 35° . The timing system was essentially the same as used previously except for a telemetry transmitter with a narrower passband, which caused a greater uncertainty in absolute time. An on-board clock was synchronised with the ground station several hours before launch and read out for each spark chamber event. The synchronisation was continuously monitored during the seven hour observation by checking the phase of the on-board clock one pulse per second against the station clock. Loran-C sky wave transmission (100 kHz) from the Yap Island station was used to monitor the absolute time of the ground station clock to 0.1 ms. VNG broadcasts from Lyndhurst, Australia, gave a check at 1 ms accuracy. All the timing signals were recorded on analogue tape. Estimated errors in the timing of events are: a systematic offset of ± 0.7 ms due to the instrumentation involved in comparing the on-board and ground station clocks; and a random error of ± 0.3 ms for

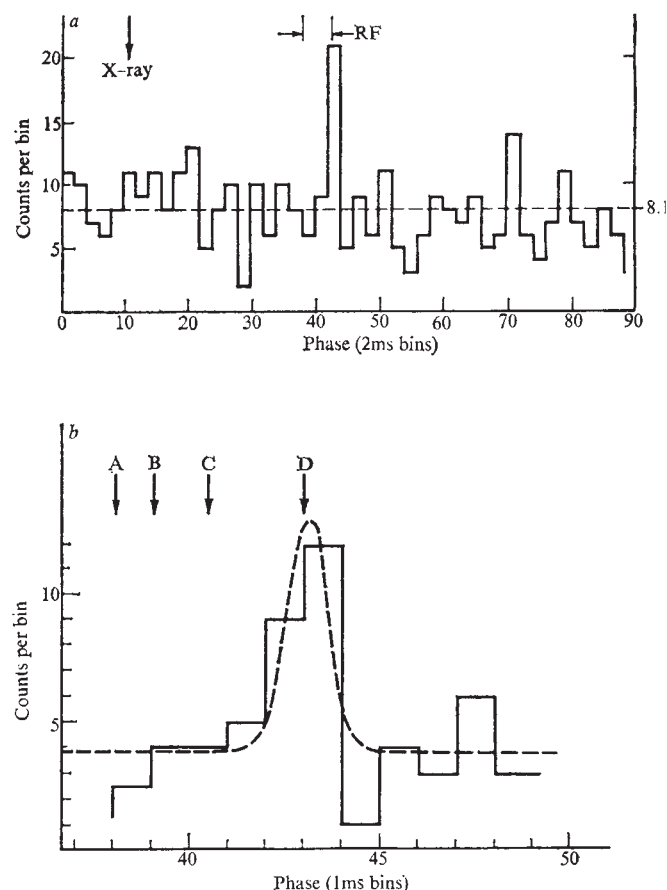


Fig. 1 *a*, Phase plot of the 10 to 30 MeV γ rays from within 15° of PSR0833-45. The X-ray phase is the observation of Harnden and Gorenstein⁴. *b*, Expanded phase plot of radio frequency and γ emission region showing radio timing markers: A, 10% amplitude, leading edge; B, 2,388 MHz peak; C, 8,415 MHz peak; D, 10% amplitude, trailing edge. — — —, Maximum likelihood Gaussian (1.2 ms FWHM).

the time of a given event, due to a combination of the clock 1/8 ms quantisation, uncertainty in balloon position, and variations in the electronics.

Figure 1a shows the phase plot in 2-ms bins of the 357 events from within 15° of PSR0833 which originated in the bottom five conversion plates of the spark chamber during the 7-h exposure to Vela. The Parkes group (unpublished work) monitored the pulsar during the week of the balloon flight and provided period and phase data at intervals of 12 min for the given time and location of the balloon. The Parkes' phase refers to the arrival time of the half-power point of the sharp leading edge of the 1,400 MHz pulse extrapolated to infinite frequency using the dispersion measure $2.8658 \times 10^5 \text{ s MHz}^2$. The absolute error on this phase is $\pm 1 \text{ ms}$. P. Reichley of JPL made similar observations and his period predictions are within several ns of Parkes' periods. The JPL phase marks the peak of the 2,388-MHz pulse. The 1-ms lag of the JPL phase is consistent with the shape of the radio pulse observed at these frequencies and the reference points on this pulse chosen by the Parkes and JPL groups.

At 5,000 MHz the pulse becomes double peaked (M. M. Komesaroff *et al.*, unpublished) with a separation of $\sim 1.8 \text{ ms}$ between the peaks. For 8,415 MHz, the later peak dominates¹³ and the early peak disappears into a slight plateau region in the early part of the pulse. Thus, the situation is more complicated than with NP0532 where the main pulse is a single sharp peak in the RF and maintains the same qualitative shape up through the infrared, visible and X-ray regions.

Figure 1b shows the various timing points in more detail. The maximum likelihood fit of the Gaussian shape to our unbinned data gives a peak position at $43.1 \pm 0.3 \text{ ms}$ and a width (FWHM) of $1.2 \pm 0.5 \text{ ms}$. In Fig. 1b this comes at the 10% amplitude point on the trailing edge of the radio pulse. The combined absolute timing errors do not preclude the γ -ray peak from being in phase with the 8,415-MHz pulse.

The statistical significance of the peak at 42 to 44 ms in Fig. 1a may be assessed from the following: With the mean of 8.1 events per bin the chances of getting 21 or more events per bin from a random distribution during the time of radio emission ($\sim 6 \text{ ms}$) is 1 in 3,000. The statistical significance of the peak is even greater if one chooses narrower bins as suggested by the 1.2 ms width of the peak from the unbinned maximum likelihood analysis. The peak is present only when events with directions near PSR0833 are chosen, and only when analysed with the unique period and change of period of PSR0833, which lends support to the identification of the peak at 43 ms as γ -ray emission from PSR0833.

A further test of the reality of the effect and determination of emission against the phase of PSR0833 may be obtained by referring to the directional data. The events from the lower five plates show a 2.5σ excess (independent of the phase of the events) at the position of PSR0833. This excess is modulated by the phase of the events. If one takes only events with phase 0–44 (time referenced as in Fig. 1a) the excess is 3.6σ . For phase 44–88 there is no excess (101 compared with 99). We note that the Harnden and Gorenstein observation⁴ of X-ray emission at $\sim 1 \text{ keV}$ occurs 29 ms before the radio emission (phase = 11 ms in Fig. 1a). Although we see no significant narrow pulse at this position, the phase plot of Fig. 1a has 179 events for phase 0–42 and 150 for the background phase.

Thus, the 10 to 30 MeV pulse structure from Vela has similarities to that of the Crab. Each shows a sharp spike $\sim 1 \text{ ms}$ in width coincident with the radio pulse. When the poor statistics γ -ray data for the Crab are combined¹⁵, there is evidence for about 15 ms of enhanced emission following the main pulse when compared to the next 15 ms. For PSR0833 our data raise the intriguing possibility that

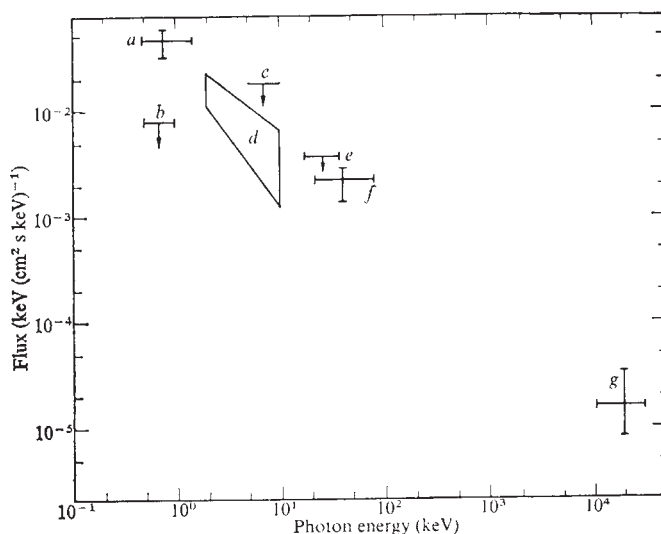


Fig. 2 Spectral data on time averaged flux from PSR0833-45 above 0.5 keV. All the points and upper limits are pulsed measurements except Uhuru (ref. 9). a, ref. 4; b, ref. 8; c, ref. 6; d, Uhuru⁹; e, ref. 7; f, ref. 5; g, present work.

a similar enhancement occurs for the 40 ms before the main peak.

The flux in the main peak to within a factor of two is $1.5 \times 10^{-5} \text{ MeV cm}^{-2} \text{ s}^{-1} \text{ MeV}^{-1}$ at 20 MeV compared to our result of $(6 \pm 3) \times 10^{-5} \text{ MeV cm}^{-2} \text{ s}^{-1} \text{ MeV}^{-1}$ for NP0532. Sturrock¹⁴ predicts a $T^{-4.25}$ dependence for the γ -ray luminosity of the pulsar on its period, that is $L(\text{Crab})/L(\text{Vela}) = 68$. Taking the Crab¹⁶ and Vela² distances as 2.0 and 0.5 kpc our measurement is 64 ± 32 . (The intensity ratio is known more accurately than the absolute intensity of either source and its uncertainty arises primarily from the statistics and the threshold setting of the Čerenkov detector.) It will be of interest to test further the Sturrock period-luminosity dependence by searching for γ -radiation from other short period pulsars with the COS-B satellite to be launched next year or the larger area balloon detectors now under construction.

Figure 2 shows the existing results on the PSR0833 energy spectrum. They are consistent with the spectral index of -0.9 observed for the main pulse of the Crab over the same energy range¹¹, but not with the -0.5 dependence predicted by Sturrock for electron polar zone radiation¹⁴. If the excess in bins 0–42 (Fig. 1) represents an actual pulsed component, the total pulsed emission (to within a factor of two) would be $4 \times 10^{-5} \text{ MeV cm}^{-2} \text{ s}^{-1} \text{ MeV}^{-1}$ at 20 MeV.

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Collimation of auroral particles by time-varying acceleration

It is generally accepted that charged particles aligned with the magnetic field, which occur in some forms of auroral precipitation¹⁻⁴, are collimated within a few thousand kilometres of the Earth's surface, by an acceleration parallel to the geomagnetic field. Static electric fields are thought to provide this acceleration, and various modes of injection of charged particles have been invoked to account for the details of observed energy spectra and angular distributions¹⁻⁴. A time-varying acceleration of an incident stream of charged particles, provides an alternative explanation.

We assume that the incident stream of charged particles has a Maxwellian energy spectrum. Such spectra are commonly observed in the plasma sheet⁵—the probable source of most auroral particles—and the energy distributions of auroral particles often resemble accelerated Maxwellian distributions. We also assume that the intensity of the incident particles is isotropic, and that there are no upward-moving particles. The intensity at energy E_1 and pitch angle α_1 , is then

$$J_1(E_1, \alpha_1) = A(E_1/E_0) \exp[-E_1/E_0] \text{ for } 0 \leq \alpha_1 \leq 90^\circ \quad (1)$$

$$= 0 \text{ for } \alpha_1 > 90^\circ$$

where A is a constant, and E_0 is the energy equivalent to the temperature of the Maxwellian distribution.

We shall first consider the energy, Δ , gained by a particle traversing an acceleration region to be constant, positive and independent of energy. The energy E_2 , and intensity, $J_2(E_2, \alpha_2)$, of the same particles measured at a detector beyond the region, follows from the energy gain and conservation of phase-space density, along a trajectory (ref. 6, and E. C. Ray, unpublished).

$$\text{Thus } E_2 = E_1 + \Delta \quad (2)$$

$$\text{and } J_2(E_2, \alpha_2)/E_2 = J_1(E_1, \alpha_1)/E_1 \quad (3)$$

The pitch angle, α_2 , follows from an assumed conservation of magnetic moment:

$$(E_2 \sin^2 \alpha_2)/B_2 = (E_1 \sin^2 \alpha_1)/B_1 \quad (4)$$

where B_1 is the magnetic field strength where the particles enter the acceleration region, and B_2 is the magnetic field strength at the detector. Combining equations (1)–(4) we have:

$$J_2(E_2, \alpha_2) = A(E_2/E_0) \exp[-E_2/E_0] \exp[\Delta/E_0] \text{ for } \alpha_2 \leq \alpha_c \quad (5)$$

$$= 0 \text{ for } \alpha_2 > \alpha_c$$

$$\text{where } \sin^2 \alpha_c = (B_2/B_1)(1 - \Delta/E_2) \text{ for } \Delta \geq E_2(1 - B_1/B_2) \quad (6)$$

$$\text{and } \alpha_c = 90^\circ \text{ for } \Delta \leq E_2(1 - B_1/B_2)$$

These results have been discussed in detail by several authors (refs 1–3, and F. W. Berko, and R. A. Hoffman, unpublished), and the main features are that accelerated particles have energies of Δ or more, and are confined to pitch angles of α_c or less, the intensity being independent of pitch angle in this range. Equation (6) shows that α_c increases with increasing E_2 and B_2/B_1 , and that maximum collimation ($\alpha_c = 0$) occurs for particles of energy Δ . Some particles back-scattered from the atmosphere would be reflected in the acceleration region and contribute to the emerging stream energies and pitch angles 'forbidden' to the particles which have traversed the region¹³.

As an alternative to constant acceleration, we postulate that Δ varies with time, with the probability of occurrence, $P(\Delta)$, of a particular value given by the normal distribution:

$$P(\Delta) = (1/\sigma\sqrt{2\pi}) \exp[-(\Delta - \mu)^2/2\sigma^2] \quad (7)$$

where μ is the mean value of Δ , and σ the standard deviation. We assume that significant changes occur in Δ within the resolving time of our detector (10^{-2} s) so that only an average effect is observed. The time scale of changes in Δ must, however, be greater than the local gyroperiod (near the Earth $\sim 10^{-6}$ s for electrons) for the magnetic moment to be conserved. Similar considerations apply to the scale size. If changes in Δ were accompanied by scattering, phase-space density would not be conserved and a different treatment would be required. For this analysis we assume, however, that equations (3) and (4) are valid, so that the observed intensity, $J_2(E_2, \alpha_2)_{obs}$, is the average of $J_2(E_2, \alpha_2)$, given by equation (5), for all values of Δ :

$$J_2(E_2, \alpha_2)_{obs} = A(E_2/E_0) \exp[-E_2/E_0] \int_{-\infty}^{\Delta_m} \exp[\Delta/E_0] P(\Delta) d\Delta \quad (8)$$

where $\Delta_m = E_2(1 - (B_1/B_2)\sin^2 \alpha_2)$, is the largest value of Δ making a nonzero contribution to the integral; which follows from equation (6). Substitution for $P(\Delta)$, and integration gives:

$$J_2(E_2, \alpha_2)_{obs} = \frac{1}{2} A(E_2/E_0) \exp[-E_2/E_0] \exp[\mu/E_0 + \sigma^2/2E_0^2] (1 + \text{erf } x) \quad (9)$$

where

$$x = [(E_2/E_0)(1 - (B_1/B_2)\sin^2 \alpha_2) - (\mu/E_0 + \sigma^2/E_0^2)]/2(\sigma/E_0) \quad (10)$$

When $\mu = 0$ and $\sigma = 0$ the particle stream is unchanged as equation (9) reverts to equation (1); when μ is nonzero and $\sigma = 0$, equation (9) reverts to equation (5), the equation for acceleration by a static electric field. On putting $\mu = 0$ with σ nonzero, random fluctuations in Δ produce a net change in intensity, but in this case equation (9) does not revert to equation (5). Equation (9) shows on inspection that the intensity increases monotonically towards smaller pitch angles (except when $\sigma = 0$) for any value of E_2 . A particularly interesting property of equation (9) is that the postulated fluctuations in Δ

can, under some circumstances, collimate both electrons and protons at the same time. An increase of the ratio B_2/B_1 will reduce the degree of collimation.

Using a sounding rocket flown during the expansion phase of an auroral substorm, we observed electrons collimated towards the direction of the local geomagnetic field. Electron energy spectra were measured three times per second over the range 0.5–10 keV, within a pitch angle range 5°–25° scanned in a 12-s period. In addition, electron intensities at 6 keV were measured every 10^{-2} s, within a pitch angle range of 20°–70° scanned every 2×10^{-1} s. Electron intensities were found consistently to increase monotonically towards small pitch angles at energies both above and below the peak of the energy spectrum.

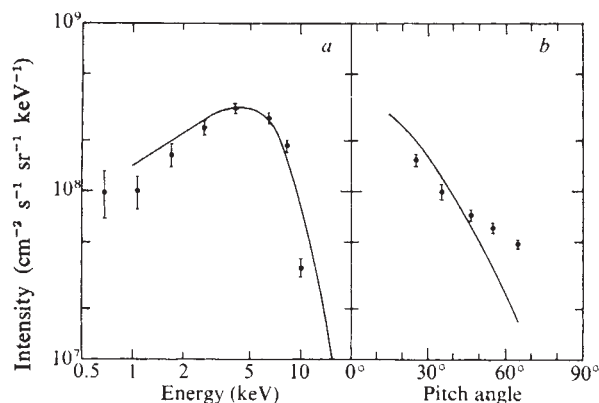


Fig. 1 *a*, Energy spectrum at a pitch angle of 20°, *b*, simultaneous pitch angle distribution at 6 keV. Measurements taken at 20.11.24 UT, on November 22, 1971, at an altitude of 135 km, using ESRO sounding rocket C78/2, launched from Kiruna, Sweden. Curves predicted using equation (9), with parameter values: $\mu=0$ keV; $\sigma=4$ keV; $E_0=1$ keV; $A=8 \times 10^7$ cm² s⁻¹ sr⁻¹ keV⁻¹; $B_2/B_1=1.33$. The ratio B_2/B_1 was chosen assuming that acceleration took place within 700 km of the atmosphere.

Figure 1*a* shows an observed energy spectrum, with a peak intensity at 5 keV and Fig. 1*b* shows the simultaneously observed angular distribution at 6 keV; typically, the intensity increases monotonically towards small pitch angles. Only 10% of the increase between 65° and 25° can be accounted for by atmospheric absorption. This was evaluated using an identical payload in a control experiment performed under stable auroral conditions, and is consistent with calculations made by Walt⁷. The absence of sharp changes in the slopes of the energy spectrum and angular distribution, together with collimation at energies below the peak of the energy spectrum are not readily accounted for by acceleration in a static electric field. Either the 'back-scattered' particles would have to be collimated before reflection, which does not agree with observations^{8,9}, or charged particles would have to be injected or produced over a range of energies at all altitudes where the electric field was present⁴. To account for our data, this injection or production would need to depend on altitude in a manner which has not been observed. On the other hand, the angular distribution and energy spectrum predicted using preliminary values of the parameters in equation (9) provide promisingly good fits to the measurements shown in Fig. 1*a* and *b*. Equation (9) also predicts the observed collimation above and below the peak of the energy spectrum.

We are unable to propose a physical mechanism which would result in the postulated distribution of the energy gained by particles. A form of stochastic acceleration¹⁰⁻¹², or an acceleration by electric fields maintained by a time-dependent anomalous resistivity could possibly induce the effect. This work was carried out as part of the research programme of the Appleton Laboratory.

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Anomalous lunar plagioclase composition and the composition of coexisting melt

THE anomalous composition of lunar plagioclases may be accounted for by various substitutions, principally the replacement by Fe and Mg, with some Ca and Na, of Al in tetrahedral sites in the lattice¹.

The composition of Apollo 11 and 12 plagioclases with less than 95% anorthite becomes increasingly anomalous as the ratio Ca/(Ca+Na) decreases (Figs 1 and 2 and ref. 2).

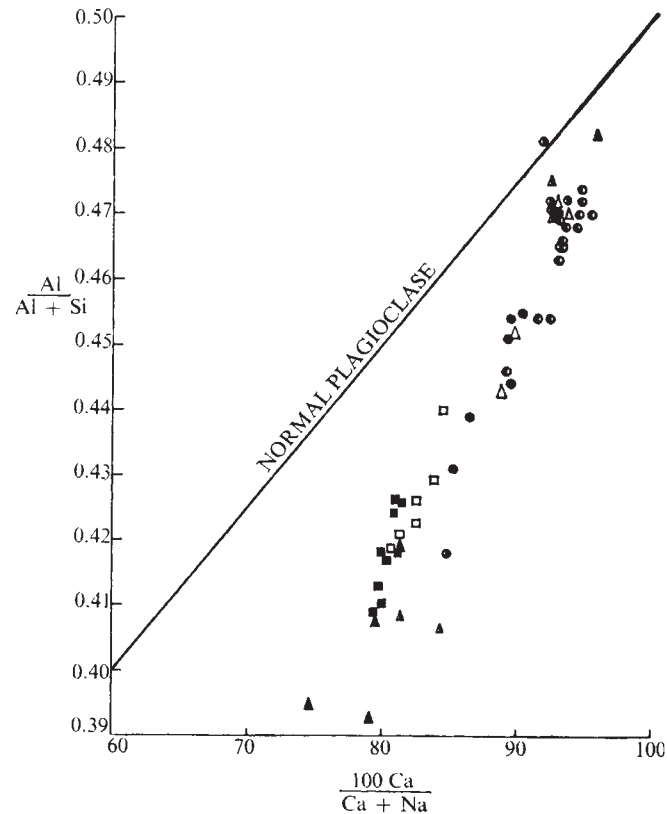


Fig. 1 Al/(Al + Si) against Ca/(Ca + Na) for Apollo 11 plagioclase. ■, 10017 (this work); □, 10017 heated at 1,108° C for 17 h in Mo, $f_{O_2} \approx 10^{-13}$ bar (this work); △, 10045 (refs 9 and 10); ▲, 10022 (refs 11 and 12); ●, ○, three lithic fragments in 10019*; ○, lithic fragments in 10021*; ▲, 10062 (this work). *Analyst Mr. P. G. Hill.

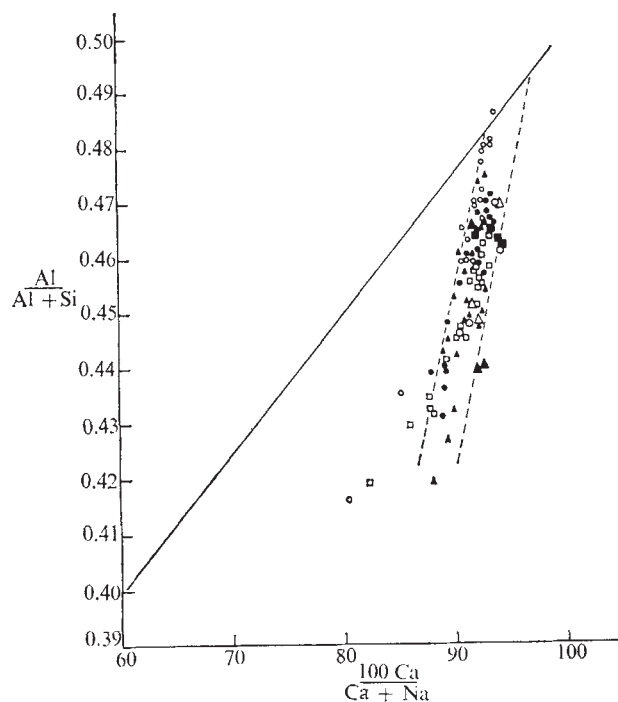


Fig. 2 $\text{Al}/(\text{Al} + \text{Si})$ against $\text{Ca}/(\text{Ca} + \text{Na})$ for Apollo 12 plagioclases. $\triangle$, 12040*; $\blacksquare$, 12040 heated at $1,149^\circ\text{C}$ for 5 h in Mo, $f\text{O}_2 \approx 10^{-12.5}$ bar (this work); $\circ$, 12040 (this work); $\triangle$, 12020 (this work); $\circ$, 12040 (ref. 13); $\square$, 12051 (this work); $\bullet$, 12051 (ref. 13); $\blacktriangle$, 12018 (ref. 13). Plagioclases in rocks 12021 and 12004 (ref. 13), 12063 (ref. 14), and 12039 (ref. 15), also plot within the area bounded by the dashed lines.

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Initially, the variation from the ideal composition is almost directly proportional to the albite content. Rock 14310, in which all plagioclases, regardless of size or albite content, have nearly ideal compositions, (Fig. 3) is exceptional. Terrestrial groundmass and phenocryst plagioclases which have a similar $\text{Ca}/(\text{Ca} + \text{Na})$ ratio to lunar basalts, exhibit no compositional anomalies. It is improbable that anomalous plagioclase is produced by the selective subsolidus volatilisation of alkalis in the lunar vacuum. Plagioclase from a Reunion basalt showed negligible change in composition after heating for 1 week at $1,000^\circ\text{C}$ *in vacuo* (10^{-7} torr). The relatively high albite content, and the complete exposure of the plagioclase surface to the vacuum in this experiment, would be more favourable to alkali volatilisation than is the case with lunar plagioclase, which has a generally low albite content and in which the surface of the plagioclase in the rock is either partially or totally covered.

It has been suggested that one of the main factors influencing the composition of lunar plagioclase is the low viscosity of the melts which, combined with rapid cooling, gives rise to rapid disequilibrium crystal growth¹. Phase equilibria studies designed to re-equilibrate lunar plagioclases with coexisting melts indicate, however, that this is not the case. Various lunar samples were heated within their melting intervals, and were then quenched rapidly (the conditions are indicated in the relevant figures). That had no effect on the anomalous nature of the plagioclase composition (Figs 1 and 2). Furthermore, there is no obvious correlation between the degree of compositional abnormality and rock texture⁴ (Figs 1–3) which is assumed to reflect the cooling rate and, thus, the degree of supersaturation and rate of crystal growth.

There is, however, a strong correlation between the variation from the ideal composition of lunar plagioclase and the compositions of the melts from which they crystallised. This is particularly apparent with the Apollo 12 rocks (Fig. 2). These show a similarity, regardless of the whole rock chemistry, between the compositions of the plagioclases and those of the coexisting melts (see Figs 3 and 4 and Table 5 of ref. 3). Plagioclases with similar albite contents exhibit similar variations from the ideal composition. The plagioclases from Apollo 11 rocks have not been as extensively investigated as those from Apollo 12 rocks but in support of my findings, the small number of determinations available in the literature indicates a similar correlation between the composition of the plagioclase and that of the coexisting melt⁵ (Fig. 1). The plagioclases in the low potassium-high titanium group of Apollo 11 rocks—10045, 10062, 10019/15, 10021/62—(refs 5–7), are similar in composition and in the degree of variation from the ideal composition, to the plagioclases from Apollo 12 rocks. So in both cases there are similarities (if titanium is excluded) between the compositions of the melts which coexist with the plagioclases and the plagioclases themselves (Table 2 and analyses 6 and 11–13 of ref. 5).

The maximum variation from the ideal composition corresponds to a deficiency of approximately 0.05 per unit formula in the $\text{Al} + \text{Si}$ content, when the $\text{Ca}/(\text{Ca} + \text{Na})$ ratio is about 0.82–0.83. That is found in plagioclases of the high potassium-high titanium group of Apollo 11 basalts—10017 and 10022 (refs 6 and 7)—and in the rims of some plagioclases from Apollo 12 rocks—12040, 12051. It seems to be the maximum deficiency acceptable to the plagioclase lattice, because it does not increase further as the $\text{Ca}/(\text{Ca} + \text{Na})$ ratio continues to decrease. The general trend with Apollo 11 and 12 plagioclases is, therefore, towards increasingly anomalous composition, reaching a limit in the Apollo 11 high potassium-high titanium basalts as the alkali content of the coexisting melts increases and the alumina content falls.

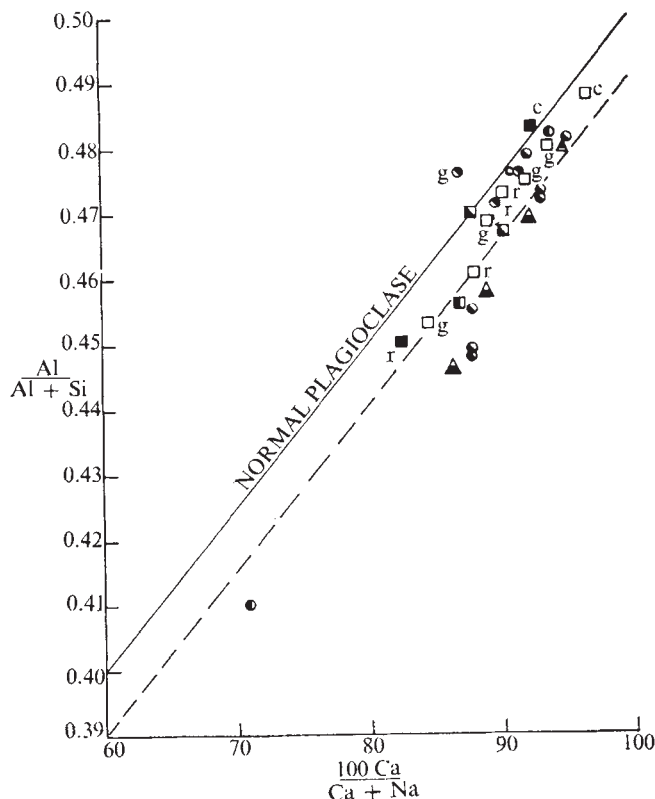


Fig. 3 $\text{Al}/(\text{Al} + \text{Si})$ against $\text{Ca}/(\text{Ca} + \text{Na})$ for plagioclases in lunar rock 14310. $\circ$, Experimental¹⁶; $\triangle$, experimental¹⁷; $\square$, $\bullet$, plagioclase analyses from ref. 18. c, Core; R, rim; g, groundmass; dashed line, maximum deviation from normal composition in terrestrial plagioclase.

The importance of the alumina content relative to the alkali content of the coexisting melt in determining the degree of variation from the ideal composition is illustrated by rock 14310. The alkali contents of the melts which coexisted with the plagioclases in that rock are similar to those in Apollo 11 high potassium-high titanium basalts, but the alumina contents are much higher—30 weight % (ref. 16) compared with 10 weight % (ref. 5)—and the plagioclases have nearly ideal compositions (Fig. 3).

If the composition of the melt is of primary importance in determining the plagioclase composition, then the same relationship should occur in lunar rocks 15555 and 15016, which have the same composition but sharply contrasted textures⁶.

I have also considered whether anomalous plagioclase compositions could result because plagioclase hosts have been analysed together with submicroscopic inclusions of olivine, pigeonite, augite and silica, or liquid. That would tend to increase Ca/(Ca+Na) ratio relative to the Al/(Al+Si) ratio and so the composition would vary from the ideal. The areas of the plagioclase crystals which I have analysed were, however, microscopically free of inclusions. If submicroscopic inclusions of olivine, pigeonite, augite or liquid are present then the extent of the compositional variation should be proportional to the Ti content of the plagioclase, which should increase as the composition becomes less ideal. This was not observed. Inclusions of a silica phase are unlikely to be present.

The anomalous composition of lunar plagioclases probably reflects the nature of the structural groups and, therefore, the activities of the ions, particularly Al³⁺, in the coexisting melts. The influence of alkalis and/or water may be critical in this respect, and it is probable that their higher concentration in terrestrial melts is responsible for the ideal composition of terrestrial plagioclases. In cases where the plagioclases have grown rapidly from melts, inherent abnormalities in composition may be exaggerated by disequilibrium crystal growth.

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Earthquake prediction and oriented microcracks in rocks

VARIATIONS in the ratio of compressional wave velocities and shear wave velocities, presage some earthquakes¹. The ratio first decreases, but just before the earthquake it returns to, or rises above, the original value. An attractive model has been proposed by Nur². Briefly, he suggested that anelastic expansion of the rock (dilatancy) reduced the velocities. With the subsequent rise of the pore pressure of water, the velocity increases again. The increased pore pressure also weakens the rock, resulting in an earthquake. All that effects the velocity of compressional waves more than the velocity of shear waves, giving the apparent variation in their ratio.

Aggarwal *et al.*³ have found, however, instances in which there are no observable variations in travel times before earthquakes. They have also observed cases in which there was a clear decrease in velocity. McEvilly and Johnson⁴ found no variations in velocities before earthquakes in central California, although variations have been detected by Robinson *et al.*⁵.

In earthquake prediction it is important to understand the underlying reason for the contradictory findings. Possibly, it is because of a lack of velocity anomalies in certain regions³. McEvilly and Johnson⁴ proposed that either the anomalous source regions for earthquakes on the San Andreas Fault in central California were too small, or the stress in these regions was below the level necessary to cause dilatancy. Nur *et al.*⁶ suggested that the dilatancy hypothesis may not be applicable to strike-slip faults such as the San Andreas. Although these suggestions offer useful explanations for particular cases I can suggest a unified explanation based on laboratory studies and theoretical predictions of the elastic properties of rocks containing oriented dilatant microcracks.

Gupta⁷ has reported experimental results on sound velocities and their ratios in a limestone subjected to anisotropic stresses: oriented dilatant microcracks can be generated by clamping a rectangular specimen in one direction and increasing the difference in stress in the other two directions. Gupta found little variation in sound velocities and their ratios along directions parallel to the cracks, but he found large variations perpendicular to that direction. Anderson *et al.*⁸ showed theoretically that parallel to the microcracks the pore fluid has little effect on sound velocities and their ratios, but that it significantly affects these properties perpendicular to the microcracks.

Nur *et al.*⁶ suggested that dilatant expansion of the crust occurs only in the direction perpendicular to the Earth's free surface. That is because expansion in that direction is opposed only by gravity, whereas expansion in the horizontal directions is resisted by the surrounding rocks. Although dilatancy in rock occurs at very high confining pressures, it is reasonable to expect that dilatant cracks in

the crust would open predominantly in the direction of least resistance. I assume the model of dilatant cracks lying parallel to the free surface of the crust. The conflicting observation of the behaviour of the precursor, can then be explained.

Figure 1 shows schematically a region of dilated crust with horizontal cracks, parallel to the Earth's surface. Five cases are illustrated. In case A, seismic waves are generated at a surface source which, together with a nearby seismic station, are located above the dilated region. This situation is similar to one of the Blue Mountain Lake experiments³ in which nearby road blasts were used as seismic sources. The ray path for the seismic waves reaching the recording station penetrates the dilated crust at a shallow depth. This is partly because of the surface location of the source and partly because of the anisotropy in

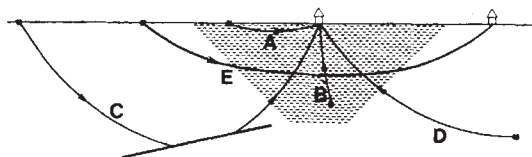


Fig. 1 Seismic rays travelling through a dilated crust. Dilatant cracks are shown as broken lines parallel to the Earth's free surface. A-E, see text.

velocity, that is, waves travelling in the horizontal direction are much faster than those in the vertical direction. Therefore, the seismic rays are strongly diffracted upward. Thus the waves follow a path nearly parallel to the Earth's surface, that is, parallel to the horizontal cracks. So neither the seismic velocities nor their ratios will show much change as cracks open and close or as the pore pressure varies.

In case B (Fig. 1), seismic waves are generated from small earthquakes in the dilated region beneath the recording station. The seismic ray traverses the dilated region at a large angle to the alignment of the dilatant cracks. Thus the opening and closing of cracks and changes of pore pressure in the cracks would have significant effects on the seismic velocities and their ratio. The situation is similar to the Garm experiments and the Blue Mountain Lake experiments¹. Seismic rays arriving at the recording station may also traverse the cracks at a large angle to their alignment if the station is above the dilated region and if the waves are either refracted from a high velocity layer below the recording station as in case C (Fig. 1) or if the waves are generated by small earthquakes outside the dilated region as in case D (Fig. 1). The latter case is similar to the situations in the San Fernando earthquake of February 9, 1971 (ref. 1) and the Bear Valley earthquake of February 24, 1972 (ref. 5). In these cases (C and D) the velocities of seismic waves will be affected by the opening and closing of the dilatant cracks and by changes in the pore pressure in the cracks, but as the ray path in the dilated region is only a portion of the total traverse, the relative change in travel time will probably not be as pronounced as in case B. Besides, in case D, the anomalous nature of source region may constitute an additional complication for the detection of the dilatant effect of the region. Finally, in case E, both the recording station and the seismic sources are outside the dilated region. Even though the seismic ray penetrates the dilated region it may traverse the region in a direction parallel to the alignment of the dilatant cracks. Therefore, neither seismic velocities nor their ratio will show much change as cracks open and close, or as pore pressure in the cracks varies. This case may be compared with the situation in the study of quarry-blast records in central California⁴.

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Crystal structure of I-tetragonal boron

THERE are three polymorphs of crystalline boron. α -Rhombohedral boron is the simplest form. It has only one B_{12} icosahedron, the basic building block in the unit cell of all crystals rich in boron. The parameters of the unit cell are: $a = 5.057 \text{ \AA}$; $\alpha = 58^\circ 04'$; $Z = 12$.

β -Rhombohedral boron has a more stable configuration, but the crystal structure is complex^{2,3}. The unit cell parameters are: $a = 10.145 \text{ \AA}$; $\alpha = 65^\circ 17'$; $Z = 105-108$.

The crystal structure of the third polymorph, II-tetragonal boron, is not known, but its unit cell parameters are: $a = 10.12 \text{ \AA}$; $c = 14.14 \text{ \AA}$; $Z = 192$.

Here, we discuss a non-metal boride known as I-tetragonal boron, which was first prepared in crystal form in 1943 (ref. 4), and which was subsequently believed to be a polymorph of elementary boron; we have accumulated results that show unequivocally that this is not the case. The crystal structure of

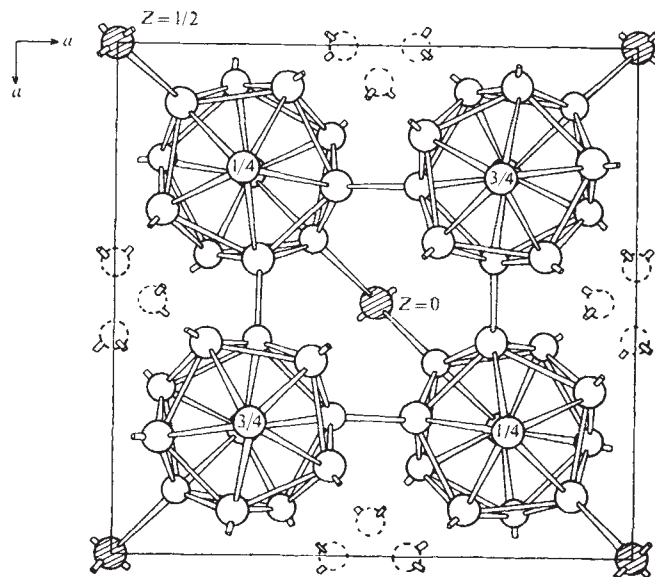


Fig. 1 Unit cell of tetragonal $B_{48}B_2C_2$ and $B_{48}B_2N_2$ projected along the c axis and including the bonding possibilities of the single boron atoms (dashed circles) distributed statistically around 4(b) (the borons in 8(h) are omitted here). Hatched circles, carbon and nitrogen atoms.

Table 1 Coordinates and occupation of the atomic sites in $B_{50}C_2$ in the space group $P 4_2/nmm$

Atom	Equivalent position (from International Tables)	No. of electrons per site	x/a	Coordinates y/b	z/c
Icosahedral atoms					
B(1)*	16(n)	5	0.3259 (2)	0.0890 (2)	0.3990 (6)
B(2)	16(n)	5 } Taken as	0.2260 (2)	0.0806 (2)	0.0873 (5)
B(3)	8(m)	5 } constant	0.1227 (5)	0.1227 (5)	0.3798 (8)
B(4)	8(m)	5 }	0.2474 (6)	0.2474 (6)	0.5849 (7)
Intericosahedral atoms					
B(5)	2(a)	0.64 ± 0.08	0	0	0
B(6)	8(i)	0.49 ± 0.03	0.4330 (37)	0	0
B(7)	8(h)	0.56 ± 0.03	0.0	0.5	0.8557 (60)
C	2(b)	5.45 ± 0.07	0	0	0.5

*Standard deviations are given in parentheses.

I-tetragonal boron has been discussed by Hoard *et al.*⁵; it has unit cell parameters: $a = 8.75 \text{ \AA}$; $c = 5.06 \text{ \AA}$; $Z = 50$.

We have prepared crystals of the non-metal borides $B_{48}B_2C_2$ and $B_{48}B_2N_2$, and have made precise electron density determinations of them.

Quantum mechanical considerations, using the concept of closed shell type boron icosahedra⁶, explain why elementary boron cannot be prepared in the I-tetragonal form. Each boron atom has four valence orbitals, one 2s and three 2p. In the LCAO approach the unhybridised set of 48 atomic orbitals of one B_{12} icosahedron can be combined into twelve equivalent outward pointing sp-hybrid orbitals and a set of 13 bonding and 17 antibonding molecular orbitals, separated by a substantial energy gap. The latter set, with the 13 orbitals, lies mainly within the icosahedron, with strong bonding forces to the next borons on the icosahedral surface. The 12 sp-hybrid orbitals are approximately perpendicular to the nearly spherical icosahedral surface, thus providing single radial sp-hybrid orbitals, each of which can form a strong σ bond between the icosahedron and the next icosahedron or any external group.

To obtain a more stable crystal configuration two conditions have to be fulfilled. First, to achieve a closed shell configuration, 38 valence electrons are required for each icosahedron. The 12 boron atoms can, however, contribute only 36 electrons and the additional electrons have to come from intericosahedral atoms. Second, to form covalent bonds, the 12 sp-hybrid bonds must find atomic orbitals close enough and in the proper directions.

It is obvious, that the structure proposed by Hoard *et al.*⁵ with the composition $B_{50} = (B_{12})_4B_2$, cannot fulfil either of these requirements.

We therefore used careful chemical preparative techniques to reproduce the crystals described by Laubengayer *et al.*⁴. These systematic investigations^{7,8}, however, yielded no results, and we concluded that it was impossible to prepare the I-tetragonal phase, $B_{48}B_2$, of elementary boron. We did succeed however, in developing methods for producing the lattice of the I-tetragonal form, using pyrolysis of BBr_3 - CH_4 - H_2 mixtures on Ta substrates yielding boron carbides⁹. This method is analogous to the method described by Laubengayer *et al.* At $1,200^\circ \text{C}$ a boron-rich tetragonal boron carbide of composition $B_{25}C$ (lattice constants: $a = 8.722 \text{ \AA}$; $c = 5.080 \text{ \AA}$; with density = 2.43 g ml^{-1}) was deposited, and pyrolysis of BBr_3 - N_2 - H_2 mixtures on BN substrates at $1,400^\circ \text{C}$ yielded a boron-rich tetragonal boron nitride of composition $B_{25}N$ (lattice constants: $a = 8.646 \text{ \AA}$; $c = 5.127 \text{ \AA}$; with density = 2.46 g ml^{-1}) (ref. 10).

We have determined subsequently the single crystal structure of both the B-C and B-N phases (ref. 10, and Will, G., and Kossobutski, K. H., unpublished). With high precision intensity measurements we were able to determine absolute electron densities in the intericosahedral positions of the icosahedral framework using Fourier sections and least-squares techniques, reaching R factors of 5.8% and 5.5% for $B_{50}C_2$ and $B_{50}N_2$ respectively. It was thus possible to

localise and differentiate between carbon or nitrogen atoms and the single boron atoms, and to assign them to well defined sites in the icosahedral framework.

The unit cell (Fig. 1) contains 50 boron atoms and two carbon or nitrogen atoms. Forty-eight boron atoms form four B_{12} icosahedra centred in the position 4(e) ($\frac{1}{4}\frac{1}{4}\frac{1}{4}$, $\frac{3}{4}\frac{3}{4}\frac{3}{4}$, $\frac{1}{4}\frac{3}{4}\frac{3}{4}$, $\frac{3}{4}\frac{1}{4}\frac{1}{4}$), which is in agreement with the crystal structure of Hoard *et al.*⁵. The remaining boron, carbon and nitrogen atoms take positions between the icosahedra in such a way that the already mentioned conditions for a stable crystal structure are fulfilled as closely as possible. The resulting chemical formulae of the crystals are then $B_{48}B_2C_2$ and $B_{48}B_2N_2$.

Within the icosahedra we found boron-boron distances of 1.774 – $1.859 \text{ \AA}$ in $B_{48}B_2C_2$, and 1.777 – $1.885 \text{ \AA}$ in $B_{48}B_2N_2$, with average B-B distances of $1.809 \pm 0.004 \text{ \AA}$ and $1.808 \pm 0.004 \text{ \AA}$, respectively. These are very similar to the values found in elementary boron^{1,3}, AlB_{10} (ref. 11), C_4AlB_{24} (ref. 12), and rhombohedral boron carbide, $B_{13}C_2$ (Will, G., and Kossobutski, K. H., unpublished).

Of the 12 icosahedral boron atoms, six form bonds with the next icosahedra, four of which have B-B distances of $1.67 \text{ \AA}$ ($B_{48}B_2C_2$) and $1.66 \text{ \AA}$ ($B_{48}B_2N_2$). Of the remaining six, two are bonded to carbon or nitrogen atoms in the position 2(b) ($00\frac{1}{2}$, $\frac{1}{2}\frac{1}{2}0$), respectively, with a B-C distance of $1.63 \text{ \AA}$ and a B-N distance of $1.59 \text{ \AA}$, and four are bonded to two single boron atoms in the statistically occupied positions 8(i) and 8(h). The B-B distances to the atoms in 8(i) and 8(h) are $1.622 \text{ \AA}$ and $1.728 \text{ \AA}$, respectively, for $B_{48}B_2C_2$ and $1.611 \text{ \AA}$ and $1.702 \text{ \AA}$, respectively, for $B_{48}B_2N_2$.

The 2(b) positions deserve special attention. They form bridges with covalent bonding between four icosahedra. These bridges are, however, occupied by carbon or nitrogen and not by boron atoms, as suggested by Hoard *et al.*⁵. The four icosahedra surrounding the C or N atoms form a flattened bisphenoid because the tetragonal c/a ratios are 0.58 and 0.59 , respectively. The boron atoms in the positions 2(a) merely fill interstitial holes. They have no bonding functions.

The main difference from former proposed structures is found in the two single boron atoms per unit cell—which give rise to the improvement of the electronic properties—and which are statistically distributed in the large tetrahedral holes around the positions 4(c) ($0\frac{1}{2}0$, $\frac{1}{2}00$, $0\frac{1}{2}\frac{1}{2}$, $\frac{1}{2}$). These tetrahedral holes measure about $3.4 \text{ \AA}$ in diameter and are therefore too large to accept just one bonding boron atom in a fixed position as a link between four icosahedra. The borons are therefore displaced towards two of the next icosahedra, which finally yields a statistical distribution over 16 positions—8(i) and 8(h), (see Table 1). The displacement is about $0.6 \text{ \AA}$ from the centre. The spatial situation easily permits such a displacement of atoms from the central position. The geometrical bonding conditions in the icosahedral framework in fact force the borons to such displacements. These additional atoms do, however, allow the conditions for a stable structure to be fulfilled to a very high degree.

The attachment of carbon, nitrogen and boron atoms to the

listed intericosahedral sites is unequivocal. The quantitative occupations given in Table 1 were derived from least-squares calculations. If, for example, carbon in (00 $\frac{1}{2}$) is replaced by boron, the *R* index increases from 5.8% to 7.5%.

Thus, the quantum mechanically unstable B₄₈ lattice is stabilised by boron and carbon or nitrogen atoms yielding the compositions B₄₈B₂C₂ and B₄₈B₂N₂. The extra atoms fill holes in the tetragonal icosahedral framework. Apart from electronic reasons the instability of the basic B₄₈ lattice also results from geometrical problems which arise from the characteristic packing difficulties in three-dimensional network icosahedral structures.

Further phases with the lattice of the hypothetical I-tetragonal boron include BeB₁₂ (ref. 13) and AlBe(B₁₂)₂ (ref. 14), and the non-metal borides B₅₀C₂ and B₅₀N₂. All can be prepared in a reproducible manner in which the tetragonal icosahedral framework is stabilised by foreign atoms. For BeB₁₂ and AlBe(B₁₂)₂, however, no absolute electron densities are known in the icosahedral holes and no further comparison is possible.

We therefore believe that the I-tetragonal boron, B₄₈B₂, is not a polymorph of elementary boron. Hoard and Hughes¹⁵ have already expressed doubts about the existence of this phase.

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Gothenburg magnetic 'flip'

SHORT events are only recorded occasionally in stratigraphical sequences, for example, during periods of rapid sedimentation, or if the record is continuous or samples are very closely spaced. If the chronology is not very exact, even these occasional records cannot usually be reliably correlated, making it difficult to distinguish between 'noise' and real events (see ref. 1).

A 'Late Weichselian magnetic reversal'² has been found in several areas of the world. We originally named³ this reversal 'the Gothenburg Reversed Event'. This name, however, may not be entirely relevant. The word 'event' is generally only applied to dipole reversals of durations of more than 10³ yr. The Gothenburg Reversal is a very short magnetic 'flip', which may need a term of its own, instead of 'event'. Magnetic 'excursions' are defined as being caused either by dipole tilting or by dipole intensity decrease with non-dipole activity becoming dominant. The Gothenburg 'flip' could be either a very short magnetic event or a magnetic excursion.

The Gothenburg 'flip' was first recognised in a core (B873) taken in the Botanical Gardens, Gothenburg²⁻⁴. The upper boundary was fixed very precisely at the boundary between the Fjärås Stadial and the Bölling Interstadial, dated at 12,350 yr BP^{4,5}. The change from reversed to normal polarity is very rapid (Fig. 1), occurring within a couple of years. Furthermore, there is no major intensity change related to the polarity change.

Since we first recognised the 'flip', we have measured several additional cores and sections (Fig. 2). Other workers have undertaken similar investigations elsewhere and the 'flip' has been found in several localities (Fig. 2).

In the samples from localities 1-15 (Fig. 2) the chronological control indicates that the recorded reversals refer to the same magnetic event or excursion, but in samples from the remaining localities the dating is not sufficiently precise for direct correlation.

As yet we do not know the exact duration and form of the 'flip'. There are three possibilities: first, a long, constant reversal which lasted 1,000-2,000 yr (the records do not, however, show a constantly negative polarity of that duration); second, a long period (1,000-2,000 yr) of unstable geomagnetism, with several flips between normal reversed polarity; third, a short period (≤ 100 yr) of reversed geomagnetism at around the Fjärås Stadial (12,400-12,350 yr BP⁴) which followed a millenium of irregular (but not fully reversed) magnetism.

Consequently, it may not be the proper time to decide whether the 'flip' should be classified as an event of very

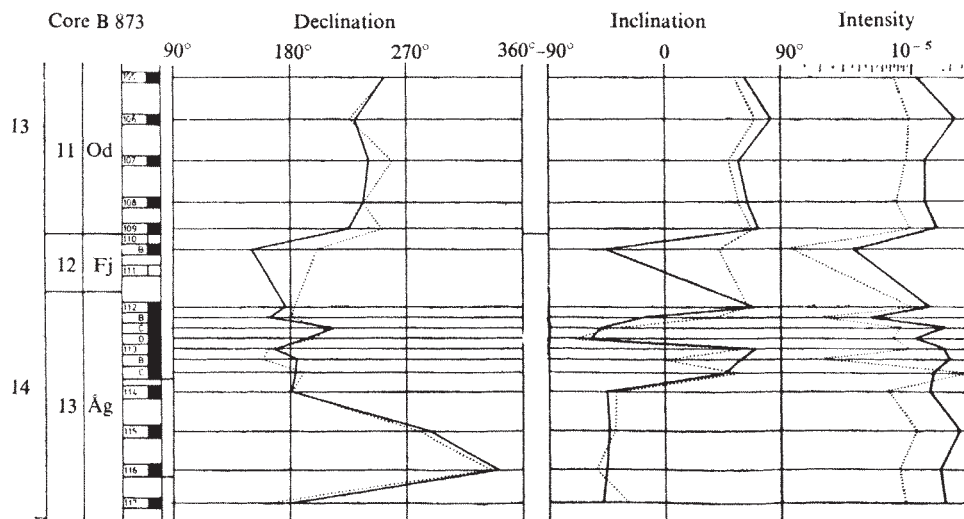


Fig. 1 Palaeomagnetic measurements of the base of Core B873 (ref. 4). The 'flip' includes layers 12 and 13, that is, the top of the Agård Interstadial (Ag) and Fjärås Stadial (Fj). Solid lines, natural remanent magnetisation; dotted lines, magnetisation after cleaning in 200 oersted. Layers 12 and 13 probably only represent about 100 yr.

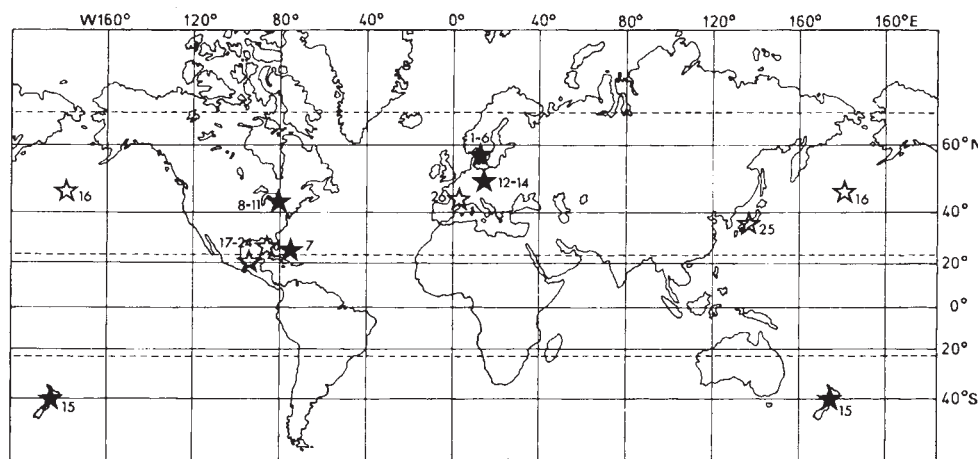


Fig. 2 Localities at which the Gothenburg geomagnetic 'flip' has been established and well dated (black stars), and at which it has been tentatively recognised (open stars). 1-6, Gothenburg²⁻⁶ and five other Swedish cores all having a well established chronology and all closely correlated on other grounds than the magnetic results (N.-A. Mörner and J. P. Lanser, unpublished); 7, deep-sea core A179-15 from the North Atlantic (ref. 6, and N.-A. Mörner and J. P. Lanser, unpublished); 8-11, four Canadian clay sections (refs 6 and 7, and N.-A. Mörner and J. P. Lanser, unpublished); 12-14, three Czechoslovakian loess sections⁸; 15, the Okupata Tephra on North Island, New Zealand (W. W. Topping, unpublished), dated at $12,450 \pm 340$ BP⁹; 16, deep-sea core V20-108 from the northern Pacific¹⁰; 17-24, eight deep-sea cores from the Gulf of Mexico¹¹; 25, Lake Biwa-Ko, Japan¹²; 26, the well known but undated Laschamp 'Event' in France^{13,14}.

short duration or as an excursion (or as a combination of both). The fact that it seems to be a full reversal not associated with any intensity decrease and that it is found in several different areas of the globe, seem to indicate that it is a real dipole reversal despite its very short duration.

So far, the 'flip' has only been looked for carefully in a few areas. In spite of that, however, it has been established in several closely dated and mutually correlated stratal sequences. Therefore, it must be a real magnetic event or excursion (not just 'noises') that has global validity. This means that the 'flip' is a global marker which can be used as a tool for detailed correlations on a global scale⁶, and its short duration is an advantage for precise correlations.

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Geostrophic comparisons using measurements from a moored current and temperature meter

THE vertical shear of horizontal current, calculated from moored current meters, can be compared with the geostrophic shear calculated from moored temperature measurements. The results of this comparison show good agreement in sign and magnitude and indicate the validity of the geostrophic balance in the conservation of momentum.

The geostrophic balance is used by oceanographers to infer ocean currents from measurements of temperature and salinity¹. Instruments which measure temperature and pressure at very short intervals have also been used². The close relationship between temperature and salinity³ in the MODE region (28°N, 70°W) allows determinations of density from temperature measurements alone, so that moorings with several temperature recorders may serve as continuously operative hydrographic stations. Current meters on the same moorings give information about the currents which may then be compared with the temperature field.

In theory, ocean currents are usually assumed to be geostrophic and hydrostatic to the lowest order, implying a thermal wind relationship:

$$\rho_0 f (\partial V_n / \partial z) = -g (\partial \rho / \partial s)$$

where $\vec{s}$ is the direction of the line joining two moorings, and $\vec{n}$ is the direction normal to $\vec{s}$. Assuming a close relationship between temperature and salinity, so that $\rho = \rho[T, S(T)] = \rho(T)$, the thermal wind equation becomes

$$\rho_0 f (\partial V_n / \partial z) = g \alpha (\partial T / \partial s)$$

where $\alpha = -(\partial \rho / \partial T)$. An attempt has been made to use the MODE-I current meter and temperature data to examine this balance in its vertically integrated form:

$$\rho_0 f \Delta V_n = g \alpha (\partial T / \partial s) \Delta z.$$

Table 1 Geostrophic comparison

Time (day of year)	Vertical shear of horizontal current			$(g/\rho_0 f)$	Geostrophic shear $\int_{-721}^{-421} \alpha(\partial T/\partial s) dz (\text{cm s}^{-1})$
	ΔV_n (cm s ⁻¹) mooring 3*	ΔV_n (cm s ⁻¹) mooring 6†	ΔV_n (cm s ⁻¹) average		
100	-6.15	-4.86	-5.51		-3.26
104	-6.31	-4.82	-5.57		-5.15
108	-4.74	-4.30	-4.52		-4.87
112	-5.23	-5.38	-5.30		-5.30
116	-6.27	-4.28	-5.27		-4.82
120	-6.66	-1.66	-4.16		-4.97
124	-7.37	-5.93	-6.65		-6.13
128	-6.18	-7.58	-6.88		-5.86
132	-6.20	-5.65	-5.92		-5.56
136	-4.79	-2.39	-3.59		-4.54
140	+0.26	-0.50	-0.12		-3.30
144	-1.77	-4.85	-3.31		-3.15

* 28°09'N 70°08'W
† 28°42'N 70°16'W

In the MODE field experiment current meters at depths of approximately 425 m and 725 m on each mooring, measured horizontal velocity and temperature. In addition a recorder at 525 m on each mooring measured pressure and temperature. In that analysis 4-d averages of both velocity and temperature were used.

Current meters on moorings approximately 60 km apart at positions 28°09'N 70°08'W and 28°42'N 70°16'W, were used to calculate the vertical change in the horizontal velocity, normal to the line joining the moorings, over the 300 m depth interval. The velocity difference for each mooring and an average velocity difference for the two moorings are given in Table 1.

To estimate the horizontal temperature gradient the temperature measurements had to be corrected to standard pressures, chosen at 421, 521, and 721 dbar. The corrections were made using the pressure measurements and a spatially averaged CTD station, giving temperature and salinity as a function of pressure (R. Milland, and H. Bryden, unpublished). It was then assumed that the pressure surfaces were essentially level, so that differences of temperatures between the two moorings at each depth could be used to obtain estimates of horizontal temperature gradients, $\partial T/\partial s$. Values of $\alpha = -(\partial \rho/\partial T)$ were obtained for each depth from the average CTD station, and the quantity

$$(g/\rho_0 f) \int_{-721}^{-421} \alpha(\partial T/\partial s) dz$$

was estimated by assuming $\alpha(\partial T/\partial s)$ to be constant over a depth interval.

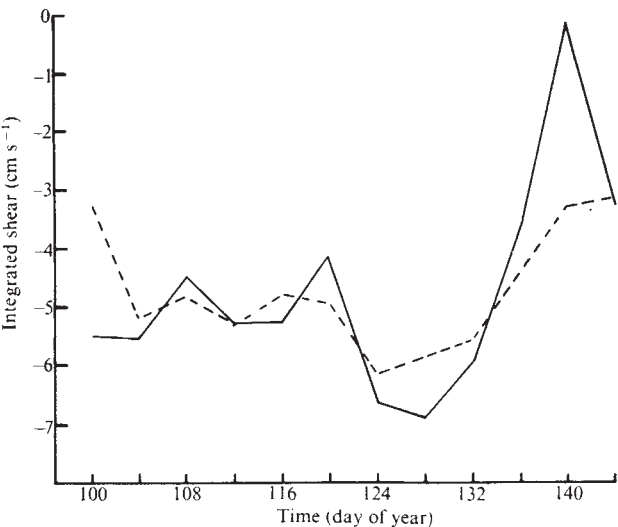


Fig. 1 Horizontal current shear, ΔV_n , from moored current meters (—), and geostrophic shear $(g/\rho_0 f) \int_{-721}^{-421} \alpha(\partial T/\partial s) dz$ from moored temperature measurements (---).

Values of average ΔV_n and $(g/\rho_0 f) \int_{-721}^{-421} \alpha(\partial T/\partial s) dz$

shown in Table 1 are plotted in Fig. 1 as a function of time. The average difference between the observed and calculated shears is 0.01 cm s⁻¹, and the average absolute difference is 0.87 cm s⁻¹, about 18% of the current shear.

Similar comparisons were attempted using other mooring pairs. All other MODE pairs with good current meters and pressure-temperature recorders were separated by horizontal distances greater than 60 km. These comparisons were not as good but they did on the whole show agreement in sign, with differences in magnitude of the shears of about 3 cm s⁻¹ over the 300 m vertical separation.

The MODE experiment is supported by the Office of Naval Research and the National Science Foundation; The Office of Naval Research, and the office of the International Decade of Ocean Exploration of the National Science Foundation maintained the equipment and provided data analysis. Dr W. Schmitz and Professor C. Wunsch kindly made released current meter data and pressure-temperature recorder data, respectively. This work was supported by the Office of Naval Research. I thank Dr N. Fofonoff for encouragement.

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Spatial ability, throwing accuracy and man's hunting heritage

ATTENTION has recently been drawn¹ to the indications that spatial ability—the ability to create, maintain and mentally transform a visual image^{1,2}—has an X-linked recessive mode of inheritance in Caucasians^{1,3-8}, with males having a proportionately greater facility^{1,9-12}. At first glance, this result seems paradoxical in that such a presumably adaptive trait would not be expected to be transmitted on a recessive allele. If past selection pressures were exerted primarily on males, however in whom the spatial allele is co-dominant, the paradox would be resolved satisfactorily.

Moreover, this is an interesting focus for anthropological research because the existence of an X-linked behavioural trait in the present would constitute independent evidence for a sexual

Table 1 Means, s.d.s and correlations among the variables

	Spatial	Velocity	Concentric circle	Horizontal directional	Horizontal deviation	Vertical directional	Vertical deviation
<i>N</i> = 61	24.64	68.91	2.82	4.88	0.84	3.87	1.10
Means	9.93	10.32	0.52	0.38	0.25	0.47	0.29
s.d.							
Spatial	1.0						
Velocity	0.14	1.0					
Concentric circle	0.26	0.51	1.0				
Horizontal direction	-0.05	0.08	-0.07	1.0			
Horizontal deviation	0.0	-0.44	-0.71	-0.08	1.0		
Vertical direction	-0.11	-0.17	-0.01	0.01	-0.04	1.0	
Vertical deviation	-0.37	-0.48	-0.83	0.20	0.32	0.10	1.10

division of labour during the 99% of human history that men have been hunters. Certainly, directional orientation and memory for visual landmarks must have been required for a man to find his way back to the group. If, in addition, spatial ability were positively correlated with judgement of distance and throwing accuracy in males (for example, as regards such weapons as stones and spears), this could presumably have allowed them to hit game at greater distances, thereby making hunting less hazardous and resulting in longer lived hunters as well as more meat.

With these ideas in mind, we have collected data on spatial ability and throwing accuracy in 67 white boys, 14–16 yr of age. Data for each subject include his age, his raw score on the PMA spatial test¹³ and several measurements of his average performance over 20 successive throws of a baseball at a vertical target 30 feet away^{14–16}. The target was a section of canvas, 9 × 9 feet, on which were inscribed five concentric circles, from 1 to 5 feet in diameter, with the centre circle or bull's eye 3.5 feet from the floor. The target area was also divided by very light lines into a grid of 81 1-foot squares. Five measures of accuracy were derived for each thrown ball. (1) Concentric circle—with point values declining from 5 for a bull's eye to 0 for throws missing the outermost circle; (2) horizontal direction—differentiating right from left, scored 0.5 to 9.5; (3) horizontal deviation—the absolute distance from the central vertical column in either direction, scored 0 to 4.5; (4) vertical direction—differentiating above from below the central row, scored 0.5 to 9.5; (5) vertical deviation—the absolute distance from the central horizontal row in either direction, scored 0 to 4.5. In addition to the accuracy records, the speed of each throw was measured and converted to a velocity in feet per second^{15,16}. Subjects were

allowed to warm up according to their own individual preferences, after which they were instructed to execute 20 over-hand throws, in two bouts of 10 each, as fast and as accurately as possible.

No significant relationship to age was found for the spatial or accuracy scores. For the spatial test, which has a multiple-choice format, only the raw scores were available and we could not correct for guessing. Therefore, six subjects scoring at or below chance levels were eliminated from the analysis. The descriptive statistics for the remaining 61 subjects are shown in Table 1.

A regression analysis was performed to assess the extent to which spatial scores were a predictor of throwing performance. The results are the univariate *F* statistics on 1 and *N*–2 = 59 degrees of freedom in Table 2a. Clearly spatial ability is significantly related only to the vertical deviation and concentric circle accuracy scores. Proceeding a step further, we performed a step-down regression analysis in which dependent variables 1 to *n* are successively removed as covariates, *n* going from 1 to 5, with the *n* + 1st variable thus being regressed on the preceding *n* variables and the spatial score. Each of the step-down *F*s then has 1 and *N*–*n*–1 degrees of freedom¹⁷. This analysis indicates that when the effect due to vertical deviation accuracy is allowed for, concentric circle scores lose their relationship to spatial ability. The converse is not true however. When concentric circle accuracy scores are partialled out (Table 2b), spatial scores still predict vertical deviation scores at the 3% level of significance. Thus, concentric circle scores show a relationship only because of their vertical component.

This relationship between spatial ability and vertical accuracy is especially interesting in that vertical deviations on an upright

Table 2 Statistics for regression analysis on PMA spatial test

(a) Variable	Square mult. R	Univariate <i>F</i> 1 and 59 d.f.	Chance probability	Step-down <i>F</i>	Chance probability
Vertical deviation	0.14	9.22	0.004	9.22	0.004
Concentric circle	0.07	4.14	0.046	0.54	0.46
Velocity	0.02	1.10	0.30	0.05	0.82
Horizontal deviation	0.0	0.0	0.98	0.38	0.53
Horizontal direction	0.0	0.13	0.72	0.14	0.71
Vertical direction	0.01	0.82	0.37	0.35	0.55
(b) Concentric circle				4.14	0.046
Vertical deviation				5.25	0.026

target would translate into skill and judgement with respect to distance if the target were laid on the ground. Therefore, while emphasising that only 14% of the variance is accounted for by the PMA test, we believe we have tentative evidence relating spatial skills to an important component of the hunting enterprise. Although caution is warranted in extrapolating from the present data to our hunting heritage, the suggestion of Washburn and Lancaster¹⁸ should be noted:

"The Pleistocene way of life can only be known by inference and speculation . . . As we go farther back in time, there is less evidence and the biological and cultural difference becomes progressively greater. Yet it was in those remote times that the human way of life took shape, and it is only through speculation that we may gain some insights into what the life of our ancestors may have been."

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Local suppression of alpha activity by pattern in half the visual field

If Lippold's hypothesis¹, that the alpha rhythm of the human electroencephalogram (EEG), (and perhaps other electrical signals that can be recorded from the scalp) are artefacts that result from eye movements, rather than genuine manifestations of neural activity, was found to be correct, it would seriously reduce the value of EEG and evoked potential techniques in research and clinical neurology. In the latter area, for example, considerable attention is being given to the validity of accepting an absence of EEG activity as a criterion of cerebral death. One of us (C. R. C.) has already discussed² earlier experiments that relate to the

eye-movement hypothesis. We will describe here results that are consistent with a neural explanation for the control of alpha activity, but not with an eye-movement explanation.

Briefly, Lippold's hypothesis states that alpha activity is found when the standing potential between the front and rear of the eye is modulated by eye movements (especially movements that are parallel to the optic axis) and by changes in the length, and therefore in the resistance, of the extraocular muscles. In addition, electrical activity of the extraocular muscles may contribute directly to the alpha rhythm. According to this hypothesis, the act of observing a visual stimulus reduces alpha activity by reducing tremor of the extraocular muscles.

If alpha rhythm depends on muscle tremor, visual stimulation should result in an equal reduction of alpha over both hemispheres, irrespective of which part of the visual field is stimulated. If alpha is under neural control, however, the amount of suppression of alpha in one hemisphere should depend upon which lateral half of the visual field is stimulated, as, except for a small central region, the primary pathways from the two halves of the visual field project to separate hemispheres. We find that visual suppression of alpha activity is related to the location of the visual stimulus.

The subject faced a rear-projection screen that subtended a visual angle of approximately $90^\circ \times 90^\circ$. The screen could be illuminated uniformly so that very little visual detail was visible, or a pattern could be projected on the left or right half of the screen. When a pattern appeared in half the screen, the subject was instructed to fixate in the blank half of the screen, about 1° from the edge of the pattern. Thus, when the pattern appeared on the left, only the right visual cortex received direct stimulation from the patterned area, and *vice versa*. In a preliminary experiment the pattern was a black-and-white chessboard, but the subject found this so soporific that the pattern was later changed to a series of coloured slides that were rich in visual detail (for example, interior views of the Royal Pavilion in Brighton). In another preliminary experiment, the effect on alpha suppression of varying the luminances of the patterned and blank fields was studied. No difference was found as luminance was varied from that luminance at which the pattern was first clearly visible, to the maximum luminance of the projector, nor did the relative luminances of the pattern and blank fields have any measurable effect. For simplicity, in the main experiment the patterns were projected at full intensity (about 200 cd m^{-2}).

The subjects were four right-handed males who had normal vision and fairly abundant alpha. Electrical activity was recorded bipolarly between chlorided silver electrodes in positions O_1-T_5 (above the inion, and left of centre) and O_2-T_6 (above inion, right of centre). In addition to conventional EEG recordings, the outputs of the left and right hemisphere preamplifiers were led to two bandpass filters that had quality factors of 10. The centre frequency of the filters was set at the individual subject's alpha frequency, which was measured from the EEG record while he faced the blank screen with his eyes open. The outputs from the filters were rectified and led to integrators that gave the sum of activity at the alpha frequency during 1 min recording periods. We used this signal, which includes both the amplitude and abundance of activity at the alpha frequency, as our principal measure of the effectiveness of selective visual stimulation.

Less activity was consistently recorded from the scalp over the hemisphere that was contralateral to the visual field in which the patterned stimuli were presented. Although the ratio of activity from the blank- and patterned-hemisphere derivations was never large (the maximum ratio, averaged over an experimental session, was approximately 2:1), it was highly significant, as shown by the inter-

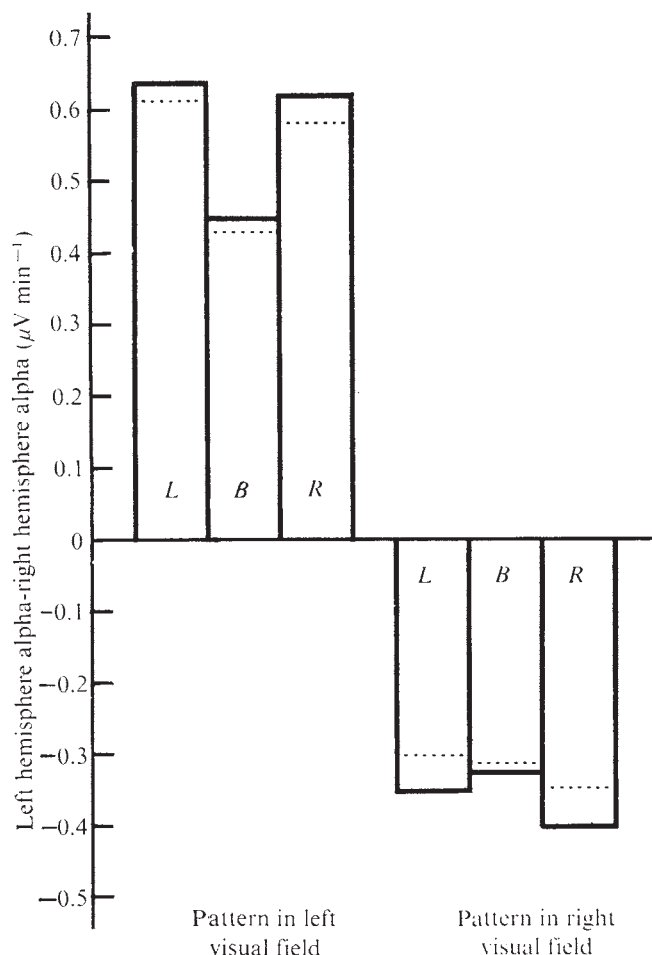


Fig. 1 Effect of left eye (L) and right eye (R) monocular observation and binocular (B) observation on the relative amount of alpha activity over the left and right occipital regions. The distance from the dotted line to the top of each bar = 1 s.e. The interaction between visual field and recording hemisphere was significant at $P < 0.00001$.

action between visual field and hemisphere (Table 1). It should be noted that this is a conservative measure of the effect of changing the side of the visual stimulation, as any extraneous electrical activity that fell within the passband of our filters, such as movement and muscle artefacts, would have added randomly to the amplitudes of the signals from both recording sites and would have tended to obscure the difference that was produced by selective visual stimulation. Table 1 also shows that the major source of variance in the recorded alpha occurred among subjects, which is not surprising. In addition, the abundance of alpha was slightly, but consistently, greater over the left hemisphere in all subjects.

Lippold suggests¹ that an important criterion for true alpha is that its frequency remain constant. We therefore monitored the frequency throughout the recording sessions by means of a digital counter that was triggered by zero crossings. Using an average response computer, triggered in the same way, we also cumulated activity from the electrodes that were ipsilateral and contralateral to the side on which the patterned stimulus was presented. Very little change in frequency was observed during periods in which alpha-like activity could be seen in the raw signal from the scalp electrodes, and at no time was there a systematic change in frequency with change in the side on which the stimulus was presented, which leads us to con-

clude that the activity that we recorded satisfies Lippold's definition of true alpha.

The principal experimental evidence that has been advanced for the eye-movement hypothesis has come from studies in which differential treatment of one eye, such as dark adaptation, or cooling of the orbit, was accompanied by the appearance of asymmetrical alpha activity over the right and left occipital regions. If alpha is controlled primarily by the ipsilateral eye, it should be predicted that asymmetrical alpha will be found when a visual stimulus is presented to one eye only. To test for this, one subject observed the visual fields monocularly as well as binocularly. (During monocular observations the other eye was covered by a flap of black cardboard.) The results in Fig. 1 clearly show that alpha activity depended on the position of the patterned and blank fields, quite irrespective of which eye was stimulated. Monocular viewing with either eye resulted in slightly more suppression than binocular viewing, but we tend to attribute this to the fact that the monocular observations were made near the end of a long recording session, when the subject had more abundant alpha, and hence more alpha to suppress.

Table 1 Results of analysis of variance of alpha blocking by visual stimulation

Source	Mean square	d.f.	P
Visual hemifield	0.73	1,144	n.s.
Recording hemisphere	31.38	1,144	$P \leq 0.00001$
Subjects	324.07	3,144	$P \leq 0.00001$
Visual field $\times$ hemisphere	31.63	1,144	$P \leq 0.00001$
Visual field $\times$ subjects	0.77	3,144	n.s.
Hemisphere $\times$ subjects	5.43	3,144	$P < 0.01$

The main findings in this study are consistent with Morrell's observation that, during an operation in which a patient's visual cortex was exposed, highly localised suppression of alpha activity could be effected by stimulating a restricted part of the patient's visual field³. All these results are explained if we accept Adrian and Matthew's conclusion that visual stimulation suppresses alpha activity by means of neural activity⁴. We do not believe that they can be satisfactorily accounted for by the eye-movement hypothesis, for in our main experiment both eyes received the same stimulation and presumably would have been induced to engage in the same movements, if indeed there were any.

These findings also strongly suggest that alpha activity is controlled by visual stimulation and not, as has been claimed, by the act of fixing the gaze⁵, or by rolling the eyes upward⁶.

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Water in the nutrient cycle of a Papuan rain forest

INTEGRATED ecosystem studies of nutrient cycling on a catchment basis¹ for tropical rain forest areas are few^{2–4}, but information on the effects of nutrient cycling in these ecosystems on soil development, hydrology, and variations in stream water quality is needed, since rain forested areas in many developing tropical countries are rapidly being cleared to provide agricultural land⁵.

I carried out an investigation into the hydrological part of the nutrient cycle in a tropical rain forested catchment of 16.26 km² below the Sogeri Plateau in Central Papua, Papua New Guinea (Fig. 1). The stream (Ei Creek) has incised approximately 600 m into the Pliocene andesitic and basaltic volcanic agglomerate capping of the plateau to expose the underlying Cretaceous phyllite, which includes chlorite, mica, and graphite, and comprises approximately 13% of the catchment. The catchment is ringed by cliffs, giving way to steep convex valley sides with a shallow covering of acid red to brown clay soils. Gullying occurs under the forest, and landslips occur after heavy rains. The rain forest of the area is dominated by *Castanopsis* and *Elaeocarpus* species which provide an open high storey, a more dense understorey, and a rich growth of ferns, epiphytes and lianes⁶.

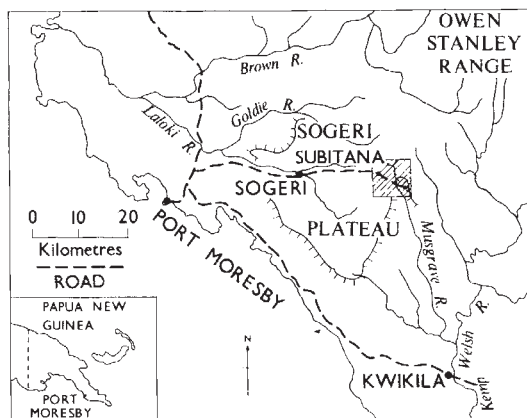


Fig. 1 Location of the study area

A Hellman type pluviograph and a Bristol gauge recorded rainfall and streamflow for 10 months (July 1972 to May 1973); samples were taken weekly, and more often when heavy storms produced stream rises. Because of human interference throughfall could not be sampled continuously, but samples were taken, through five individual storms and an incidence of dew fall, with four 0.25 m² polyurethane coated troughs placed 1 m above ground. This method provides a large sample area equivalent to approximately 75 standard 13 cm diameter polythene funnels, but tends to mask the true throughfall variability. Leaf litter leachate and soil water solutions were simulated by washing with distilled water^{7,8}.

Water samples were analysed with a Varian Techtron atomic absorption spectrophotometer for Ca, Na, K and Mg. Samples were not buffered for Ca analysis⁹, and the absence of Ca in the cation sequence (Table 1) for leaf litter leachate may be due to interference or precipitation out of solution; Ca results may therefore be anomalous.

Table 1 Cation sequences (meq l⁻¹)

Rainfall	Na > Mg > K > Ca
Throughfall	Na > Ca > K > Mg
Leaf litter leachate	Mg > Na > K
Soil water (to 15 cm)	Mg > Ca > Na > K
Soil water (to bedrock)	Mg > Na > K > Ca
Stream water	Mg > Na > Ca > K

The colorimetric heteropoly blue method¹⁰ was used for Si analysis. Dissolved salts and suspended solids in stream water were also measured.

The figures for the annual transfer of nutrients in the hydrological cycle (Table 2) are derived from measured elemental concentrations and measured or estimated volumes of water. Because of insufficient throughfall measurements an approximate value, of 80% rainfall as throughfall and stemflow, was arrived at by reviewing the literature for other tropical rain forests^{11–13}. The chemistry of stemflow has been found to be not very dissimilar to throughfall^{12,14}, and, as it constitutes a small percentage of the rainfall reaching the forest floor, its inclusion with throughfall will not lead to large errors in estimates of nutrient return.

In simulating leaf litter leachate and soil water solutions by washing with distilled water there is the problem of accounting for residence time of water in the compartments, and the lateral flow and mixing of water with depth in the soil. McGinnis *et al.*¹⁵ calculated a residence time of 0.35 d for water in the litter layer, and 33.06 d for water in the soil of a moist tropical forest. If residence time represents leaching ability, the annual turnover of nutrients in these compartments may be very different from that calculated here. The rate of nutrient turnover in the forest floor is probably dependent upon both the volume of water and the length of time the compartment remains saturated. Not all of the 2,155 mm reaching the soil surface will reach the bedrock, and use of this volume of water in calculating the nutrient flow out of the lower soil compartment may result in an overestimate. Since the soils in this study were shallow and lateral flow on the steep slopes may add to the flow, the overestimate may not be too great. In spite of these problems, the cation sequences in Table 1 are very similar to those found in lysimeter studies under rain forest in Costa Rica².

The values for throughfall in the nutrient cycle indicate the rates and relative importance of the movement of individual elements in the hydrological section of the cycle. On a kg ha⁻¹ basis, Na provides the greatest return in throughfall, followed by K, Ca and Mg. Neither Nye¹² nor Kenworthy³ measured Na in throughfall, but both found the above sequence. The relative importance of throughfall in total nutrient return (litter, timber and throughfall) was shown by Nye¹² to be: K, 75%; Ca, 9%; and Mg, 25%. Although actual amounts of Mg in throughfall are low, throughfall is of relatively greater importance to Mg than to Ca in their respective cycles. Both Jordan¹⁶, and Sollins and Drewry¹⁴ considered that in tropical rain forests nutrient return in throughfall, and in particular K, is as important as litter fall in the nutrient cycle.

Silicon was not found in rain or throughfall, but the high Si content of the forest floor is considered by Rodin and Bazilevich¹⁷ to be typical of the tropical rain forest. Si represents a different type of cycling from the other elements considered here; it is not washed out in throughfall and depends entirely on litter and timber fall for its return to the ground, whereafter it must be leached from the litter layer in order to be returned to the roots through the soil. The low pH and destructive flora and fauna of the forest floor provide an ideal environment for the removal and transfer of Si. The accumulation of Si by forests¹⁸ is of geological significance and many tropical trees have

Table 2 The nutrient cycle

	Depth water (cm yr ⁻¹)	kg ha ⁻¹ yr ⁻¹				
		Si	Ca	Na	K	Mg
a, Rain	269.37	0	0	8.35	0.81	0.27
b, Throughfall	215.50	0	23.27	83.18	34.91	6.47
c, Leached from canopy	215.50	0	23.27	74.83	34.10	6.20
d, Leaf litter	215.50	13,447.20	0	6,439.14	6,661.11	5,021.15
e, Surface 15 cm soil	215.50	512.24	232.74	234.25	333.16	983.97
f, Soil to bedrock	215.50	436.39	6.47	159.04	121.54	170.68
g, Stream water	147.83	288.08	24.75	66.00	14.93	50.95
h, Total nutrients (a+c+d+e+f+g)	—	14,683.91	287.23	6,981.61	7,165.65	6,233.22
i, Net loss (g-a)	121.55	288.08	24.75	57.65	14.12	50.68

No. samples: a, 20; b, 49; c-f, 3; g, 99.

been found to contain high concentrations¹⁸. Mg dominates the cation sequence for the litter, soils and stream water. This was found to be the case in McColl's study², and supports Kenworthy's observations³ that most of the Mg is derived from litter leachate. Litter is of great importance under tropical rain forests because of the rapid rate of decay, estimated by Nye¹² to be 1.3% d⁻¹.

In stream water, Si, Mg and Na, showed strong tendencies to dilute with increasing discharge, Ca displayed a poor dilution relationship, and K concentrated weakly with increasing discharge. Similar relationships with discharge have been found in widely differing study areas^{20,21} which suggests that biological rather than geological processes are dominant in the variability of stream water quality. The large amounts of nutrients involved in both the solid and dissolved phases of the cycle, compared with the losses in stream water (less than 1.2% of total nutrients measured), demonstrate the fully closed nature of the nutrient cycle. The persistence of relatively low concentrations of elements in stream water through a wide range of discharges is due to the efficiency of the root systems in filtering out the nutrients leached through the soil. The loss in stream water, however, does not include the elemental composition of suspended sediment and organic debris. At peak flows after large storms much sediment and organic debris is washed into the stream from the forest floor; trees are carried downstream and the large bedload is moved. In one instance, suspended sediment after a storm rose from 22.8 p.p.m. at 1.11 cumecs to 1,945.7 p.p.m. at 51 cumecs. This material is an important form of nutrient loss from the ecosystem.

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Regulation of larval corpora allata in *Galleria mellonella*

IN insects metamorphosis occurs when the corpora allata stop secreting juvenile hormone, but it is not known how the production of juvenile hormone is regulated. Wigglesworth¹ first indicated that the nervous connections from the brain to the corpora allata provide a mechanism for control; this control could be nervous or neurohormonal. In many adult insects, the corpora allata seem to be inhibited via their nervous connections to the brain, since in most cases severing these nerves activates the glands^{2,3}. Whether the same is true for larvae has not been demonstrated conclusively. Activation of the corpora allata by neurosecretion has been demonstrated in many adult insects, for example *Tenebrio molitor*⁴, *Leptinotarsa decemlineata*⁴ and *Rhodnius prolixus*⁵, and the production of an allatotrophic hormone by the larval brain has also been suggested⁶⁻⁸. We have now investigated the control of corpora allata function in last instar larvae of *Galleria mellonella*, encouraged by the discovery that in this species the implantation of brains at the beginning of the last larval instar causes an extra larval moult⁹. Our results show that the corpora allata are activated by a neurohumoral factor from the brain during larval development and that they are inactivated by nervous inhibition just before metamorphosis.

We first confirmed that the implantation of brains causes supernumerary moults, by implanting brains dissected from 48–120 h last instar larvae under the dorsolateral abdominal epidermis of water-anaesthetised hosts. During the first 48 h of the last larval instar, the hosts responded to the implantation of three brains by undergoing a supernumerary moult (Table 1). Identical results were obtained after the implantation of corpora cardiac-corpora allata or brain-cardiac-allata complexes^{10,11}. The capacity of the host larvae to respond to the implanted brains was lost about 60 h after the last larval-larval ecdysis.

Table 1 Development of last instar larvae implanted with three brains at different times after the last larval-larval ecdysis

Hosts Age at time of implantation (h)	No.	Larval-larval ecdysis		Larval-larval ecdysis	
		% of insects	Length of instar (d)	% of insects	Length of instar (d)
12	89	55	5.0	45	8.0
48	9	44	6.3	56	7.4
60	11	9	8.0	91	8.5
120	28	0	—	100	8.3

Brains came from donors 48–120 h after the last larval-larval ecdysis.

To distinguish whether the implanted brains activate the corpora allata^{12,13} or the prothoracic glands, we performed the same implantations on 0–12 h last instar larvae which had been allatectomised. The allatectomised larvae did not respond to the implanted brains, indicating that in the previous experiments the brains were primarily stimulating the corpora allata. Thus before the last larval instar, the corpora allata appear to be activated by a factor from the brain. During the last larval instar, either the brain no longer produces or releases this factor, or the glands are inhibited from responding to it.

We investigated the capacity of brains from sixth and seventh (last) instar larvae to induce extra larval moults in sensitive last instar larval hosts (Table 2). The ability of the brain to activate the corpora allata seems to change during development, being most potent during the first part of the instar and declining just before the moult. Implanted brains from 120 h last instar larvae were the least able to elicit extra larval moults. This corresponds to the period between 54 and 152 h in the last larval instar when the changes from the larval to the pupal programme of development can no longer be affected by large amounts of exogenous juvenile hormone^{11,15}. Since

Table 2 Induction of extra larval-larval ecdyses by implantation of three brains from donor larvae of different ages into 0–12 h last instar larval hosts

Age of donor	No. of larvae	% of insects forming supernumerary larvae	% of insects forming pupae
24–36 h, 6th instar	16	94	6
Premoult 6th instar	16	37	63
0–12 h, 7th instar	34	71	29
48 h, 7th instar	23	61	39
72 h, 7th instar	37	44	56
120 h, 7th instar	16	91	81
Moveable prepupa*	17	35	65
Sham operated	25	0	100

* Eye pigment stage 3–14 (ref. 14).

brains from 0–72 h last instar larvae can induce an extra larval instar when implanted into sensitive hosts, while the corpora allata lose their sensitivity to implanted brains about 48–60 h after the last larval-larval ecdysis, reactivation of the corpora allata *in situ* might be prevented by nervous stimuli. We have found that severing the nerves to the glands 72 h after the last larval-larval ecdysis partially restores their activity (Table 3), since two of our experimental animals moulted into supernumerary larvae, whereas no effect was seen in a comparable number of sham-operated animals. The length of the instar to the larval-pupal ecdysis in those animals which had the nervous connections severed was prolonged significantly, indicating at least some restoration of activity of the corpora allata. Since a definite, but less pronounced, prolongation of the instar was observed in the sham-operated animals, injury apparently contributes to this effect. Most significantly, severance of the nerves rendered host larvae sensitive to the implantation of brains, at a time when unoperated larvae did not respond. The fact that only 20% of these animals underwent an extra larval moult is probably due to damage caused to the glands during nerve severance. We conclude that there is probably a nervous inhibitory influence on the corpora allata which develops between 48 and 60 h after the last larval-larval ecdysis.

Since the implanted brains activate the corpora allata through the haemolymph, the activating factor appears to be of a neurohormonal nature. Further evidence for this comes from the observation that the number of supernumerary larvae resulting from brain implantation increased with the number of brains implanted (Fig. 1).

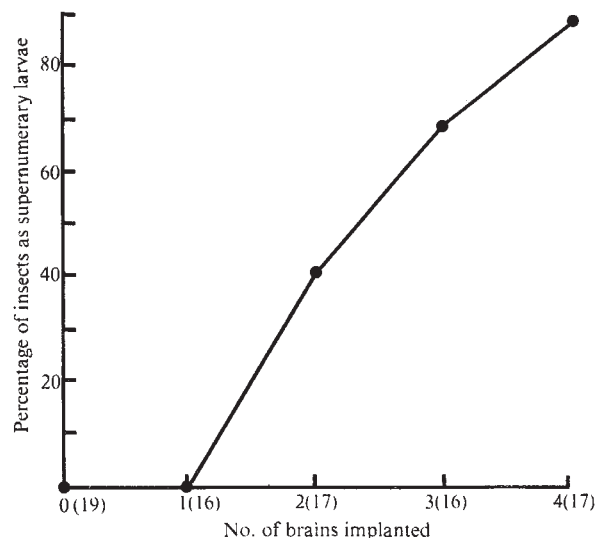


Fig. 1 Extra larval development as a function of the number of brains implanted into larvae 0–12 h after the last larval-larval ecdysis. The numbers in parentheses are the numbers of experimental animals.

The most prominent neurosecretory cells in the brain of larval *G. mellonella* comprise a heterogeneous group found in the pars intercerebralis (ref. 16 and our unpublished work). To test whether the activating factor is produced by cells in this region, we destroyed bilateral regions of the brain selectively by microcautery and implanted the cauterised brains into sensitive hosts (Table 4). Significantly fewer extra larval moults were elicited by brains cauterised in the pars intercerebralis. This suggests that the allatotrophic factor is a neurohormone produced by neurosecretory cells in the pars intercerebralis.

Our results show that in larval *G. mellonella* the corpora allata are activated by a neurohumoral factor from the brain, possibly a neurohormone produced in the pars intercerebralis. A nervous inhibitory influence on gland function probably develops between 48 and 60 h after the last larval-larval ecdysis, subsequently being reinforced by a decrease in the production or release of the allatotrophic factor by the brain.

Table 3 Development of 72 h last instar larvae after severance of the nerves innervating the corpora cardiaca-corpora allata, and implantation of brains 0–24 h later

Operation	No. of larvae	Larval-larval ecdysis % of insects	Length of instar (d)	Larval-pupal ecdysis % of insects	Length of instar (d)
Host plus three brains	20	0	—	100	7.5 ± 0.8
Nerves severed	31	7	15.0 ± 4.2	93	16.0 ± 7.7
Nerves severed plus three brains	35	20*	16.3 ± 5.5	80	13.2 ± 4.1
Sham operated	29	0	—	100	11.0 ± 3.2

Brains came from donors 48–120 h after the last larval-larval ecdysis.

* Three animals were intermediates: two were score 3; one was score 4 (ref. 11).

The regulatory mechanism for the control of the function of the corpora allata is undoubtedly more complex than the situation we have described. Juvenile hormone has been reported to stimulate the secretion of ecdysone by the prothoracic glands^{17,18}, and the corpora allata appear to be influenced by ecdysone, whose production by the prothoracic glands is under the control of neurohormones

Table 4 Induction of extra larval-larval ecdyses by implantation of brains cauterised in different regions in 0-12 h last instar larvae

Operation	No. of larvae	% of insects forming supernumerary larvae	% of insects forming superlarvae
Sham cautery	18	72	28
Cautery area 1	24	25	75
Cautery area 2	19	63	37
Cautery area 3	19	58	42
Cautery area 4	20	50	50
Cautery area 5	20	53	47

Each host was implanted with three brains from 0-12 h last instar larvae. Area 1, pars intercerebralis; area 2, corpora pedunculata; area 3, anterior to corpora pedunculata; area 4, lateral to corpora pedunculata; area 5, medial to tritocerebrum.

from the brain¹⁸. The brain obviously plays a central role in these relationships, and experiments are under way to investigate the possibility of endocrine feedback mechanisms in the brain.

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Cyclic GMP and cyclic AMP levels in normal and transformed fibroblasts

THE 3'-5' cyclic nucleotides, cyclic AMP and cyclic GMP have been implicated in the control of the growth of cultured fibroblasts in that early, transient decreases in intracellular cyclic AMP¹⁻³ and increases in cyclic GMP⁴ are observed on reinitiation of the growth of quiescent mouse fibroblasts by serum. Moreover additions of high, nonphysiological concentrations (10^{-5} M to 10^{-3} M) of either cyclic AMP or cyclic GMP (or their butyrate analogues) either suppress the induction of DNA synthesis after serum addition⁵ or initiate substantial increases in DNA synthesis respectively¹ in quiescent mouse fibroblasts. Cyclic nucleotide concentrations in asynchronously-growing cell cultures are an average over the entire cell cycle and depend for cyclic AMP on the proportion of cells in a particular phase. The average cyclic AMP level is often correlated with growth rate⁶, although this may not be such a reliable para-

meter for cell growth as the relative proportions of G1, S, G2 and M vary with cell type and the culture conditions⁷. The larger changes in cyclic GMP are correlated directly with the initiation of fibroblast growth when cells are moving out of the G0 state or by passage through the M and G1 boundary and are not exhibited during the remainder of the cell cycle (W. S., and P. S. R., unpublished). Thus cyclic GMP concentrations and the ratios of cyclic AMP to cyclic GMP may be more sensitive and reliable indicators of the growth state of a cell.

Here we demonstrate that nontransformed fibroblasts show a large difference in cyclic GMP and cyclic AMP content and cyclic AMP:cyclic GMP ratios for growing and quiescent fibroblasts; no such differences occur in transformed fibroblasts. These results suggest fundamental differences in the control of cyclic GMP concentrations between normal and transformed fibroblasts in monolayer cultures.

Addition of fresh medium and 20% serum to quiescent cultures of Swiss mouse 3T3-4A fibroblasts caused early, transient increases in cyclic GMP and decreases in cyclic AMP concentrations, reaching maximum and minimum levels after 10 to 20 min, respectively (Table 1a), similar to the changes observed in quiescent BALB/c 3T3 cultures⁴. The concentration of cyclic nucleotides present in a cell is more accurately expressed relative to the total protein content rather than the DNA content or the number of cells as both the total protein and the volume of a cell change in harmony during the cell cycle⁸. Prostaglandin E₁ (PGE₁) elevates intracellular cyclic AMP concentrations in 3T3-4A cultures by a stimulation of adenyl cyclase activity¹. Addition of PGE₁ with the new medium caused increased levels of cyclic AMP, suppressed the rise in cyclic GMP observed after 20 min, and reduced the induction of DNA synthesis by a third. An increase in cyclic AMP presumably inhibits the cyclic GMP increase as PGE₁ does not directly affect the membrane-bound guanyl cyclase (P. S. R., and W. S., unpublished).

Table 1 Kinetics of cyclic nucleotide changes on reinitiation of cell growth

	Time (min)	Cyclic AMP	Cyclic GMP	Cyclic AMP/ cyclic GMP
<i>a</i> Serum addition to 3T3				
	0	19	0.7	28
	5	2.0	3.5	0.6
	10 (PGE ₁)*	2.4 (4.4)*	3.6 (0.5)*	0.5 (8.4)*
	20 (PGE ₁)*	3.1 (26)*	2.2 (0.5)*	1.4 (48)*
	60 (PGE ₁)*	5.8 (9.5)*	2.7 (2.2)*	2.1 (4.3)*
<i>b</i> Leucine addition to SV3T3				
	0	10.0	3.8	2.6
	5	7.2	3.2	2.2
	10	7.6	3.4	2.3
	20	7.6	4.9	1.5
	60	8.8	3.6	2.5

a, Swiss mouse 3T3-4A cultures were grown in 9-cm Petri dishes for 10 d (2.5×10^6 cells per dish)¹⁷, the medium was then replaced with fresh DEM (ref. 17) and 20% serum with* or without $10 \mu\text{g ml}^{-1}$ prostaglandin E₁ (PGE₁) and cultures were isolated at the stated times for cyclic nucleotide determinations^{4,19}. Parallel cultures were also radioactively labelled with ³H-thymidine from 18-22 h or from 8-32 h after medium change for DNA synthesis and autoradiography respectively⁴. The c.p.m. incorporated into DNA per 10^6 cells (% of cells with radioactively labelled nuclei) were 3.4×10^3 (1.2%), 200×10^3 (91%), and 90×10^3 (36%) for the original cultures, cultures with fresh medium, and cultures with fresh medium and PGE₁ respectively. *b*, Simian virus 40 transformed 3T3-4A cells (SV3T3) were grown to a density of 6.1×10^6 per dish, cell monolayers were washed twice with DEM lacking leucine, and DEM containing 1/200 of the normal leucine concentration and 5% dialysed serum was added back and incubated a further 2 d (5.5×10^6 per dish)¹². Then regular concentrations of leucine were restored and cultures were isolated as above. Cell density 30 h later was 9.9×10^6 per dish; DNA synthesis and cell cycle data are shown in Table 3. Half of each sample for cyclic nucleotide determination was digested with 3'-5' cyclic nucleotide phosphodiesterase and the values obtained (0.2-0.3 pmol for cyclic AMP and 0.6-0.9 pmol for cyclic GMP) were deducted to yield the final results^{4,14}. Duplicates agreed to within 15% and results are expressed in pmol of cyclic nucleotide per mg of cell protein²⁰.

Table 2 Cyclic nucleotide concentrations in growing, resting, and serum-activated cultures

Cell type	Growth state	Labelled nuclei (%)	Cyclic AMP	Cyclic GMP	Cyclic AMP/cyclic GMP
BALB/c 3T3 mouse (10% serum)	Growing	93	8.4	2.2	3.4
	Resting	0.4	15.4	0.8	19
	Activated	86	4.7	4.1	1.1
BALB/c 3T3 mouse (2% serum)	Growing	74	6.8	2.3	3.0
	Resting	0.5	9.9	0.7	14
	Activated	88	2.4	9.8	0.25
3T3-4A Swiss mouse	Growing	73	7.2	2.9	2.5
	Resting	0.5	16.1	0.6	29
	Activated	91	3.2	4.2	0.78
Secondary mouse embryos	Growing	79	4.5	2.3	2.0
	Resting	1.4	17.4	1.1	16
	Activated	71	3.3	10.0	0.33
BSC-1 monkey	Growing	93	7.2	2.1	3.3
	Resting	0.5	13.3	0.7	18
	Activated	95	3.4	4.4	0.77
BHK hamster	Growing	56	7.6	3.5	2.1
	Resting	2.3	13.2	1.6	8.4
	Activated	86	2.9	15.4	0.19
3T6 Swiss mouse	Growing	86	6.5	2.0	3.3
	Resting	10	9.7	0.8	13
	Activated	92	2.5	2.9	0.84
SV3T3 transformed mouse	Growing	92	8.5	4.9	1.7
	'Resting' (confluent)	84	8.6	4.5	1.9
	Activated	97	7.0	4.3	1.6
PyBHK transformed hamster	Growing	80	5.6	4.7	1.2
	'Resting' (confluent)	83	7.4	4.3	1.7
	Activated	89	6.2	4.5	1.4

Twenty Petri dish cultures¹⁷ for cyclic nucleotide determinations were isolated after 2 or 3 d on reaching a quarter to half of the final cell density (growing), after 8–9 d (resting) (3–4 d after the cessation of DNA synthesis in nontransformed cells or at confluency for the transformed cells), or after 20 min following addition of fresh DEM and 20% serum to 'resting' cultures (activated) (Table 1). The cyclic nucleotide amounts are expressed in pmol per mg cell protein and DNA synthesis by the percentage of cells with radioactively labelled nuclei after exposure to ³H-thymidine for 24 h before isolation of growing cultures or from 8–32 h after the time of medium change for resting and activated cultures. Serum concentrations and the cell densities upon isolation ($\times 10^6$) of growing and 'resting' (or activated) cultures respectively were for BALB/c 3T3 in 10% serum 0.85 and 2.2, in 2% serum 0.65 and 1.1 (serum growth activated cultures isolated after 10 min instead of 20 min); for Swiss mouse 3T3-4A in 10% serum 1.6 and 2.8; for secondary mouse embryo fibroblasts in 10% calf serum or 0.5% horse serum 2.5 and 6.4; for African green monkey kidney fibroblasts (BSC-1) in 10% serum 0.61 and 4.0; for baby hamster kidney fibroblasts (BHK) in 10% and 1% serum, 2.9 and 0.36 (confluency about 10); for Swiss mouse 3T6 fibroblasts 7.2 and 18.0; for simian virus 40 transformed 3T3-4A (SV3T3) in 10% serum, 2.6 and 12.8 (confluency); and for polyoma virus transformed BHK (PyBHK) in 10% serum, 8 and 25.2 (confluency).

The cyclic nucleotide contents of different fibroblasts were compared when cultures were randomly growing, in a quiescent state mainly as a consequence of the exhaustion of growth factors in serum⁹ or in a synchronised growing state 20 min after fresh medium and 20% serum were added to quiescent cultures (Table 2). When growing cells ceased to divide and became quiescent, cyclic AMP levels increased by about two-fold (fourfold for mouse embryos) while cyclic GMP levels decreased by a factor of about two to three. On the initiation of growth of the quiescent cultures, cyclic AMP levels fell three- to fivefold while cyclic GMP rose four- to twelvefold. Because of the parallel but reciprocal changes in cyclic GMP concentrations the cyclic AMP:cyclic GMP ratio reflected these changes more dramatically. It was lower for growing cells (2 to 4), high when cells became quiescent (12 to 30) either when in contact (BALB/c 3T3 in 10% serum) or in sparse cultures (BALB/c 3T3 with 2% serum), and about unity or below in the synchronised growth state (activated for 20 min). Baby hamster kidney (BHK) and mouse 3T6 fibroblasts which contained a significant fraction (2.3% and 10%) of cells synthesising DNA also have the lowest cyclic AMP:cyclic GMP ratios of the 'resting' fibroblasts tested. In contrast, the cyclic nucleotide concentrations for virally transformed fibroblasts SV3T3 and PyBHK did not change significantly between the three growth states (Table 2) and cyclic GMP levels were a factor of two higher than for the growing fibroblasts. Since the transformed cells failed to exhibit density-dependent inhibition of growth^{9,10}, they were actively synthesising DNA even when the cultures were confluent (Table 2).

Artificially depriving the culture medium of required nutrients is another reversible method of controlling the growth of both nontransformed¹¹ and transformed¹² fibroblasts in culture. Depending on the depleted nutrient the 3T3-4A fibroblasts cease to grow in the G1 phase or at random, whereas the transformed fibroblasts, SV3T3, only stop at random in the cell cycle.

Restoration of the depleted nutrients initiates growth and cell division. Only the 3T3-4A fibroblasts that were synchronously arrested in the G1 phase of the cell cycle by deprivation of histidine and glutamine showed significant increases or decreases in cyclic GMP and cyclic AMP:cyclic GMP ratios, respectively, 20 min after addition of the depleted amino acids (Table 3). The kinetics of the changes were similar to those shown in Table 1 on growth initiation by serum (not shown). Methionine deprivation arrested cells at random in the cell

Table 3 Reinitiation of growth in amino acid-starved cultures

Culture	Labelled nuclei (%)	Cyclic AMP	Cyclic GMP	Cyclic AMP/cyclic GMP
3T3-Met	4	7.3	2.5	2.8
3T3+Met	21	7.7	4.0	2.0
3T3-His-Glu	3	10.0	0.8	13
3T3+His+Glu	86	6.5	12.5	0.5
SV3T3-Leu	1	10.0	3.8	2.6
SV3T3+Leu	67	7.6	4.9	1.6

Swiss 3T3-4A (3T3) and SV3T3 cells were grown for 3 d in complete media (Table 1) (1.0 and 6.1×10^6 cells per dish), and then in medium containing 1/100 of the normal concentration of methionine (Met) or histidine and glutamine (His, Glu) for 3T3 (ref. 11) or 1/200 of leucine (Leu) for SV3T3 (ref. 12) (Table 1). After 3 d for 3T3 (1.8 and 1.0×10^6 cells for minus Met or His and Glu) or 2 d for SV3T3 (5.5×10^6 cells), half the cultures were supplemented with the normal concentrations of the depleted amino acids and nine or ten petri dish cultures were isolated from both the original starved cultures (–) and those cultures reinforced for 20 min with the added ingredients (+). The pmol cyclic nucleotides per mg cell protein and the % of cells with radioactively labelled nuclei after exposure to ³H-thymidine from 8–32 h after additions were recorded as in Table 1. Cell cycle results from the average DNA content per cell measured in a microfluorometer¹¹ expressed as the percentage of cells in G1, S, G2 were for 3T3-Met: 71, 12, 17; for 3T3-His-Glu: 94, 0, 6; for growing 3T3-4A (Table 2): 57, 26, 17; for resting 3T3-4A (Table 2): 94, 0, 6; for SV3T3-Leu: 38, 29, 23; for growing SV3T3 (Table 2): 45, 30, 25. (ref. 12).

cycle and no comparable changes in cyclic nucleotide concentrations were observed when it was restored (Table 3). Leucine deprivation arrested SV3T3 cells randomly throughout the entire cell cycle (Table 3) and no significant early increases in cyclic GMP levels were observed when growth was initiated by restoration of this amino acid, though cyclic AMP levels fell by about 25% after 20 min (Table 1b).

Addition of purified growth-initiating molecules, phytohaemagglutinin or fibroblast growth factor from bovine pituitary glands to quiescent cultures of human peripheral blood lymphocytes¹³ or BALB/c 3T3 mouse fibroblasts¹⁴, respectively, caused large early increases in intracellular cyclic GMP but little change in cyclic AMP. Conversely addition of PGE₁, which raised intracellular cyclic AMP, reversed the induction of DNA synthesis in quiescent 3T3-4 mouse fibroblasts and stopped growth in dividing cultures^{1,2}. That both fibroblast growth factor and PGE₁ stimulated membrane-bound guanylate¹⁴ or adenyl cyclase¹ enzymes, respectively, suggest that cyclic GMP could act as a positive while cyclic AMP acted as a negative intracellular signal for controlling the growth of cultured cells. We have shown here that cyclic GMP concentrations as well as those of cyclic AMP (refs 1 and 2) are closely correlated with the growth state of different cultured fibroblasts and that parallel but reciprocal changes are observed in the cyclic nucleotide concentrations in transition between these growth states¹⁵.

The changes in cyclic nucleotide concentrations, particularly those of cyclic GMP, are largely lost when the cells become transformed, whereas the average cyclic GMP concentrations were approximately twice as high in transformed as in non-transformed fibroblasts. The failure to observe changes in cyclic GMP for transformed cells probably reflects the failure to arrest the transformed cells in the G1 or G0 phase of the cell cycle. Whether this is caused by a lesion in the production of the putative pleiotypic programme of the cell^{16,17} or as a consequence of the failure of fibroblastic factors from delivering their growth controlling signals¹⁸ is still uncertain at present.

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Inhibition of cell growth by oxygenated derivatives of cholesterol

CELLS growing in a chemically defined, sterol-free medium must synthesise cholesterol or, in the case of L cells, desmosterol¹ for membrane formation. Studies by others showed that when cholesterol or desmosterol was added to L-cell cultures the exogenous sterol was utilised and the rate of sterol synthesis was diminished, presumably by a negative feedback mechanism^{2,3}. Our observations⁴, however, provide new information regarding the structures of sterols that affect sterol synthesis. Exogenous cholesterol and various metabolically related steroids do not inhibit sterol synthesis in mouse liver cell or fibroblast cultures under conditions where derivatives of cholesterol oxygenated in the 7, 20, 22 or 25 positions are highly inhibitory. These inhibitory sterols, at concentrations of 0.02-0.2 $\mu\text{g ml}^{-1}$ specifically depress the activity of the regulatory enzyme in the sterol synthetic pathway, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1.1.1.34), within 6 h.

We now report that prolonged incubation of L cells with the inhibitory steroids diminishes the concentration of cellular desmosterol, results in the reduction or cessation of the growth of the culture, and creates a requirement for an external supply of an appropriate sterol or for a sterol precursor further along the biosynthetic pathway than HMG-CoA.

L cells were grown in chemically defined medium⁴ in Falcon culture flasks (25 cm²) for 1-3 d before the introduction of the experimental media as described below. The sources of the sterols and procedures used to purify them have been reported previously⁴. Steroids suspended in a solution of 5% crystallised bovine albumin were added to cultures previously refed with fresh medium so that the final concentration of albumin was 0.05% or 0.1%. Control cultures were fed medium containing an equivalent amount of albumin. The cultures were incubated on a rotating table (20 r.p.m.) at 37° C and the medium was renewed every 3 d. At the end of the incubation period cells adhering to the flask were washed twice with physiological saline, digested with 0.1 N KOH and assayed for protein^{5,6}. The amount of cellular protein in a culture is a good measure of cell growth^{6,7}, at least during the log phase of L-cell culture.⁸ Our preliminary studies showed that there was a good correlation between cell number and the amount of protein or DNA in growing L-cell cultures and in cell cultures incubated with different steroids as described below.

The most potent inhibitor of cell culture growth was 25-hydroxycholesterol followed, in order of decreasing activity, by 20 α -hydroxycholesterol, 7-ketocholesterol, and 7 α -hydroxycholesterol (Fig. 1a). The first three sterols inhibited growth of cultures by more than 90% at 1, 2.5 and 10 $\mu\text{g ml}^{-1}$, respectively. In contrast to results obtained

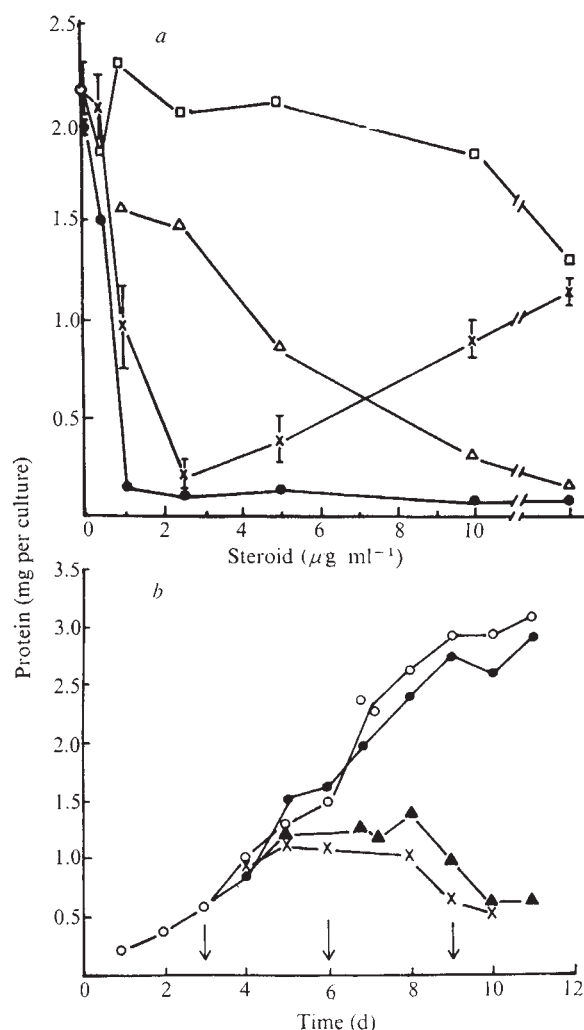


Fig. 1 *a*, Inhibition of cell growth by some oxygenated derivatives of cholesterol. Cultures were grown for 2 d in normal medium then in medium containing indicated concentrations of 25-hydroxycholesterol (●); 20 α -hydroxycholesterol (×); 7-ketocholesterol (Δ); or 7 α -hydroxycholesterol ($\square$). Cultures were refed with experimental or control media after a further 3 d and cellular protein was analysed 2 d after refeeding. Vertical lines represent range of values for at least 3 replicate flasks. *b*, Effects of 20 α -hydroxycholesterol alone or in combination with acetate or mevalonate on the time course of cell growth. Cultures were grown for 3 d in normal medium then (first arrow) in control medium (○) or in medium containing 20 α -hydroxycholesterol, 10 $\mu\text{g ml}^{-1}$ (▲); 20 α -hydroxycholesterol, 10 $\mu\text{g ml}^{-1}$ and MVA, 1 mg ml^{-1} (●); or 20 α -hydroxycholesterol, 10 $\mu\text{g ml}^{-1}$ and acetate, 1 mg ml^{-1} (×). The cultures were refed with control or experimental media on days indicated by the second and third arrows.

with the other inhibitory sterols, cell growth improved when 20 α -hydroxycholesterol was increased beyond that required for maximum inhibition, possibly due to the utilisation of this diol to meet the sterol requirement for cell growth. Under similar conditions, 7 α -hydroxycholesterol at 10 $\mu\text{g ml}^{-1}$ was not inhibitory but at 50 $\mu\text{g ml}^{-1}$ did inhibit growth by 50%. In experiments not shown, 7 β -hydroxycholesterol was more inhibitory than 7 α -hydroxycholesterol.

The relative effectiveness with which these sterols inhibited the growth of cultures correlated well with their previously reported capacities to inhibit sterol biosynthesis and to depress levels of HMG-CoA reductase activity⁴. Sterol concentrations required to arrest growth within 3 or 4 d, however, were several times higher than those necessary to inhibit sterol synthesis within 6 h. The apparent discrepancy between these values may indicate

that sterol synthesis must be almost totally blocked to obtain the marked depression in the growth of the cultures that we observed. The extent to which cell growth could be adversely affected by an inhibitory sterol also depended on the stage of growth of the culture. A growing, sparse culture was far more susceptible to inhibition than a nearly confluent, dense culture.

Figure 1*b* illustrates the time course of the inhibitory effect of 20 α -hydroxycholesterol on the growth of cultures. The amount of protein in the cultures increased for the first 2 d after the addition of the sterol, remained essentially constant for about 3 d, then declined. Supplementation of the medium with mevalonate (MVA), the product of the reaction catalysed by HMG-CoA reductase, counteracted the effect of 20 α -hydroxycholesterol and resulted in a normal rate of growth. In contrast, supplementation with acetate did not relieve the inhibitory effects of the sterol.

In other experiments the inhibitory effect of 20 α -hydroxycholesterol, 25-hydroxycholesterol or 7-ketocholesterol was abolished by the addition of desmosterol or MVA. Concentrations of desmosterol and MVA required to fully counteract inhibition were about 25 $\mu\text{g ml}^{-1}$ and 1 mg ml^{-1} , respectively. Addition of MVA or desmosterol alone to control cultures did not appreciably alter the cell growth, suggesting that, under usual culture conditions, the rate of growth was not limited by the rate of sterol synthesis. The abilities of various sterols and their precursors to counteract the inhibitory effect of a 20 α -hydroxycholesterol on the culture growth are compared in Table 1. Cholesterol was nearly as effective as desmosterol, whereas cholestanol, Δ^7 -cholestenol, cholest-4-en-3-one and cholest-5-en-3-one were moderately effective. Other sterols, HMG, or acetate, showed little or no ability to counteract inhibition by 20 α -hydroxycholesterol.

Estimates of cellular desmosterol after incubation with the inhibitory sterols alone or in combination with the various additions to the medium shown in Table 1 were made after hydrolysing the washed cells with 1 N KOH in 50% ethanol for 2 h and extracting the neutral fraction with ether. Sterols in the extracts were analysed by gas chromatography as the free sterols or after conversion to the trimethylsilyl ethers^{4,9}. After 5 d of incubation with the inhibitory sterols in amounts sufficient to inhibit cell growth by 70% or more, desmosterol concentration in the surviving cells was less than 1 $\mu\text{g mg}^{-1}$ protein, markedly reduced from that of the control cells (11.4 ± 4 $\mu\text{g mg}^{-1}$ protein). When the growth inhibitory effects of 20 α -hydroxycholesterol, or 25-hydroxycholesterol were counteracted by the addition to the medium of a non-inhibitory sterol, concentrations of desmosterol in the cells remained low

Table 1 Effect of sterols and their precursors on the growth of cells incubated with 20 α -hydroxycholesterol

Additional compounds	Cellular proteins (% of the control)
None	30 $\pm$ 4 (4)*
Desmosterol	95 $\pm$ 3 (4)
MVA (sodium salt)	92 $\pm$ 1 (4)
Cholesterol	77 $\pm$ 10 (5)
5 α -Cholestanol	68
Δ^7 -Cholestenol	67
Cholest-4-en-3-one	57
Cholest-5-en-3-one	49
5 α -Cholestan-3-one	36
β -Sitosterol	35
HMG (sodium salt)	17 $\pm$ 2 (3)
Acetate (sodium salt)	17 $\pm$ 1 (3)

All flasks except controls were incubated for 5 d with medium containing 20 α -hydroxycholesterol (10 $\mu\text{g ml}^{-1}$) and additional compounds as indicated (steroids, 50 $\mu\text{g ml}^{-1}$ MVA, HMG, and acetate, 1 mg ml^{-1}).

*Mean $\pm$ s.e. Numbers in parentheses indicate number of experiments.

If, however, the medium contained mevalonate instead of a non-inhibitory sterol, desmosterol increased to $12.8 \pm 2.6 \mu\text{g mg}^{-1}$ protein, and several unidentified sterol peaks were also present on the chromatogram. Small amounts of the inhibitory sterols and larger amounts of the non-inhibitory sterols administered with the inhibitors were detected in the cell extracts.

The results of these experiments demonstrated that L cells became severely deficient in desmosterol when incubated with certain oxygenated derivatives of cholesterol which specifically inhibit sterol biosynthesis at the level of HMG-CoA reductase. The growth of the cultures was then greatly depressed unless an appropriate sterol or sterol precursor was supplied exogenously. Several explanations of the effect of the inhibitory sterols on culture growth can be brought forward. Possibly the inhibitory sterols compete with desmosterol for sites in the membrane with resulting effects on membrane function. Specific repression of HMG-CoA reductase activity by the inhibitors would then be a coincidental and unrelated effect. We believe that it is more likely that the effects of the inhibitors on cell growth stem from repression of the enzyme activity and the subsequent restriction of sterol synthesis. Partial depletion of cholesterol from erythrocytes and mycoplasma results in increased membrane fragility^{10,11}, so that the growth retardation of L-cell cultures that were unable to synthesise sterols may have been due to membrane instability and cell death. On the other hand, it is now apparent that altering the sterol concentration of artificial cell membranes, and membranes of erythrocytes or mycoplasma changes the fluidity of the membrane, the rates at which various substances are transported through the membrane, and the activities of membrane-localised enzymes¹²⁻¹⁴. Thus, the inhibitory sterols may influence growth of the culture in ways other than by increasing membrane fragility.

For the first time the amount and kind of sterol in living mammalian cells can be manipulated through the use of specific inhibitors of sterol synthesis so that it should be possible to investigate in detail the role of sterols in membrane structure and function and their relationship to cell growth.

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Morphological and functional maturation of human thymic epithelium in culture

ORGAN culture of embryonic avian and murine thymus¹⁻⁵, as well as explants of guinea pig and rabbit thymus⁶⁻¹⁰, have been observed to produce outgrowths which in some cases were described as predominantly lymphoid in nature, epithelial in other cases. Two publications have described human embryonic thymus in culture, using the plasma clot technique^{9,10}. We have established epithelial outgrowths from explants of human thymic tissue and maintained these cultures for periods up to 3 months.

Fresh thymic tissue was obtained at surgery from 20 children, ranging in age from 1 week to 13 yr, with various surgically correctable cardiac lesions. Capsular tissue and haemorrhagic areas were dissected away and the remaining tissue was finely minced in Dulbecco's modification of Eagle's medium, containing 100 units ml⁻¹ of penicillin, 100 $\mu\text{g ml}^{-1}$ of streptomycin, and 2.5 $\mu\text{g ml}^{-1}$ of amphotericin-B, supplemented with 30% foetal bovine serum (FBS, Flow Laboratories). The preparation

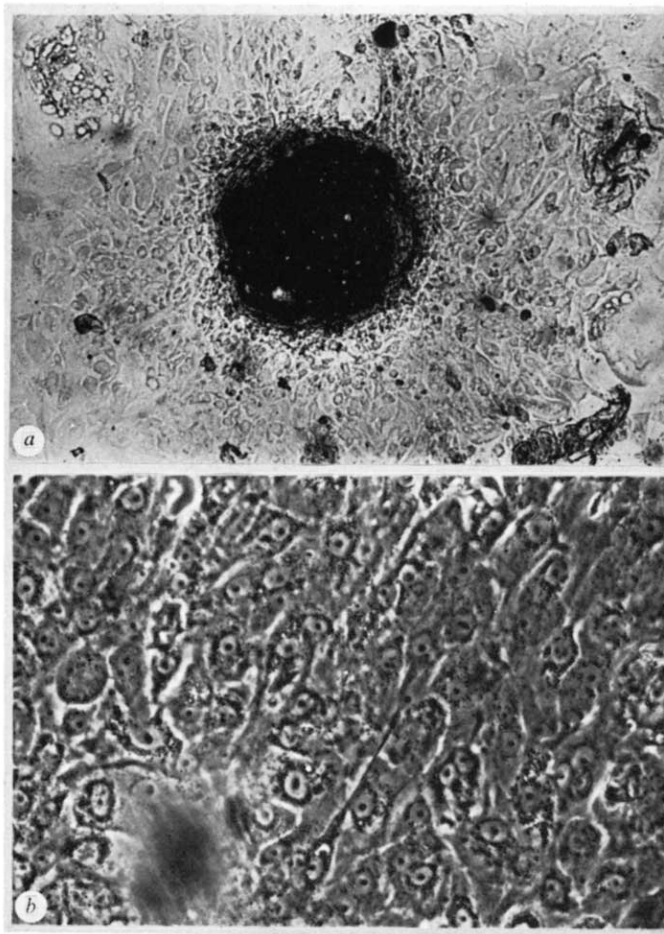


Fig. 1 Living cultures of human thymic epithelium at 30 d. a, The primary explant is present as a dense central mass with a surrounding monolayer of large epithelial cells in a mosaic-like arrangement ($\times 26$); b, phase contrast shows the cells to have a large central nucleus with a prominent central nucleolus and marked cytoplasmic granularity ($\times 104$).

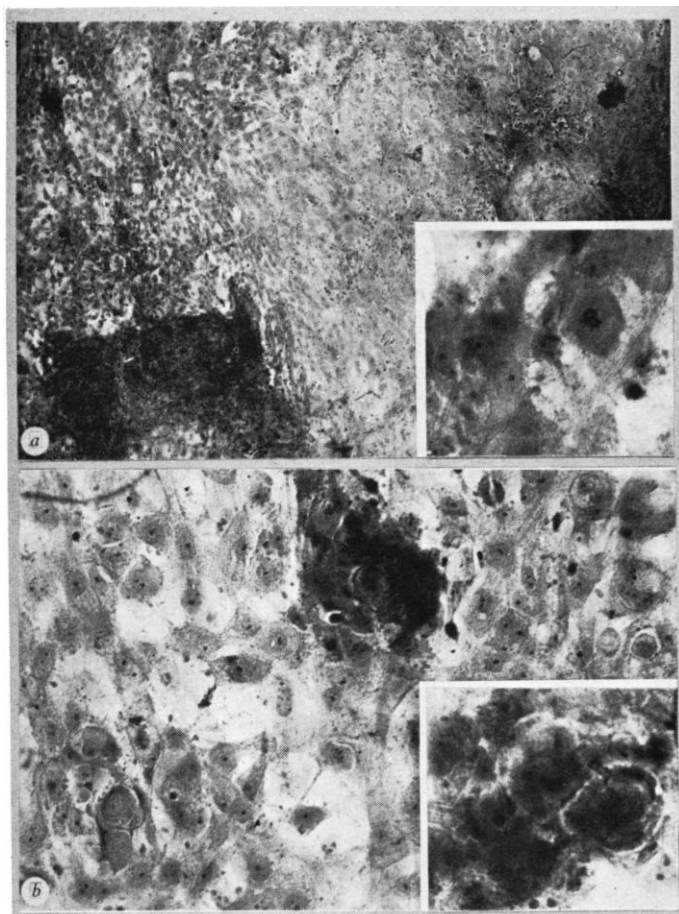


Fig. 2 Ten day culture of human thymus on glass coverslip, fixed *in situ* and stained with haematoxylin and eosin. *a*, Two primary explants are present as dense masses of dark-staining cells, with the epithelial outgrowth spreading between the two explants as sheets of large cells with abundant, somewhat granular cytoplasm ($\times 3.76$). Inset, mitotic figure at the periphery of advancing epithelial growth ($\times 60.16$); *b*, cell aggregates resembling Hassall's corpuscles in various stages of development; left lower corner: a group of cells showing early cyst formation with cell crescenting ($\times 37.60$). Inset, dense aggregate of cells arranged in concentric patterns around central amorphous eosinophilic material ($\times 60.16$).

was then washed with a threefold volume of the same medium, allowed to settle for 5 min at room temperature, and the resultant cell suspension removed by pipette. The tissue fragments remaining were washed three times by this method, then transferred to culture. In most cases the fragments, in a small volume of medium sufficient only to cover the culture surface, were added to plastic tissue culture flasks (Falcon number 3012 and 3024). In several cases, fragments were also cultured in plastic tissue culture dishes (Falcon number 3005) with and without 22 mm glass coverslips, to be subsequently fixed and stained for cell morphology. Incubation was at 37°C in a humidified atmosphere containing 5% CO_2 . On day 3, an equal volume of fresh medium was added.

By days 5–7 an epithelial outgrowth, twice the diameter of the explant, was observed. The medium was replaced twice weekly until a confluent monolayer was obtained, when the FBS concentration in the medium was reduced to 20%. Medium collected from the cultures (conditioned medium) was centrifuged at $550g$ for 10 min at 10°C and the supernatant removed and stored at -20°C . In all flasks an outgrowth of large epithelial cells from the explants was seen (Fig. 1) which produced a confluent monolayer, usually by day 14. Cells morphologically identified as fibroblasts were present in all cultures and persisted in most cases as a 5–10% contamination of the monolayer.

Explants grown on coverslips were fixed *in situ* and stained with haematoxylin and eosin. Aggregates of cells arranged concentrically around central amorphous eosinophilic material were seen scattered singly and in groups in the epithelial outgrowth, both adjacent to and remote from the primary explanted tissue (Fig. 2). These resembled Hassall's corpuscles as seen in sections of normal thymic tissue. Cultures fixed and embedded *in situ* in plastic flasks were examined with the electron microscope and showed desmosome formation between cells, confirming their epithelial nature.

Decrease in the concentration of FBS in the medium below 30% at the time of initial explantation resulted in a proportionate decrease in the rate of epithelial growth but had no effect on fibroblast growth. Consequently, cultures with 20% FBS were overgrown by fibroblasts within 30 d, while those with 10% FBS were overgrown in 14–20 d. When single cell suspensions were cultured in the same conditions, fibroblast growth predominated. Thymic lymphocytes showed a gradual decrease in number with time in culture, as shown by Wright-Giemsa staining and E rosette formation (a marker of T lymphocytes)¹¹ of cells shed into the medium from the monolayers, until none was detectable by these means at 30 d in culture.

Conditioned medium collected from the cultures and stored frozen was examined for its ability to influence the capacity of human marrow cells to form rosettes with sheep erythrocytes at 4°C (E rosettes). Marrow obtained by aspiration was separated on Isopaque-Ficoll gradients¹¹ to remove erythrocytes and enrich for lymphoid cells. Cells from the gradient interface were washed three times with phosphate-buffered saline. Some of the cells were resuspended in PBS to a concentration of 3×10^6 nucleated cells ml^{-1} and assessed for rosette formation after 2 h at 4°C (ref. 11). Rosettes were also enumerated by counting the remaining cells after incubation for 180 min at 37°C with fresh medium, conditioned medium from thymus cultures, and conditioned medium from Chang human liver cells (Flow Laboratories). The results (Table 1) suggest that conditioned media from confluent human thymic epithelial monolayers only were capable of increasing the capacity of unfractionated human marrow cells to form rosettes with sheep erythrocytes in the cold, while non-lymphoid cells (Chang human liver), peripheral blood lymphocytes, tonsillar lymphocytes, and cells from an established line of B lymphocytes were unaffected by the conditioned media. The most effective thymic conditioned media were obtained when thymic lymphocytes were no longer present in the cultures.

Table 1 Percentage of E rosettes

Test media	Marrow			Cell preparation		
	A	B	C	Peripheral blood lymphocytes	Tonsillar lymphocytes	Cultured human lymphocytes
Saline	5	17	14	50	29	0
Medium	10	12	12	37	21	0
Chang	13	13	13	49	24	0
Thymus	22	33	32	46	27	0

Bone marrow cells from three donors (A, B, and C) were separated on Isopaque-Ficoll gradients, washed three times with phosphate-buffered saline (PBS) and adjusted to 3×10^6 nucleated cells ml^{-1} . A portion of cells were resuspended in PBS and examined for rosette formation with sheep erythrocytes at 4°C (E rosettes). Other portions were resuspended in 1 ml of test medium: fresh medium containing 20% FCS; Chang: conditioned medium from confluent monolayers of Chang human liver cells, stored at -20°C ; thymus: conditioned medium from various confluent monolayers of human thymus epithelium, stored at -20°C , incubated at 37°C for 3 h, washed twice with PBS, resuspended in PBS to 3×10^6 nucleated cells ml^{-1} , and examined for formation of E rosettes. Rosettes were evaluated after 2 h at 4°C , and are expressed as a % of nucleated cells present. The capacity for formation of E rosettes of the control cells was unaffected by the thymus culture conditioned media, but the marrow cells showed an increase in E rosette formation with these conditioned media.

A differentiation process within the thymus of lymphocytes originating from stem cells in haematopoietic tissues is central to the concept of a distinct thymus-derived or T-cell population of circulating lymphocytes¹². Evidence for cell-free thymic differentiation factors has been based on reconstitution *in vitro* and *in vivo* of several immune functions and T-cell associated markers in thymectomised and nude mice, using extracts of fresh bovine and murine thymic tissue¹³⁻¹⁷. The epithelial stroma of the thymus has been suggested as the source of such differentiation factors¹⁸. Our observations suggest that an epithelium of apparent thymic nature may be obtained *in vitro* from human tissues and that this epithelium may be a source of a factor or factors with biological activity similar to those extracted from fresh bovine and murine thymus.

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Asbestos induces selective release of lysosomal enzymes from mononuclear phagocytes

THE association of asbestos inhalation and lung disease has been recognised for many years¹. Asbestosis is a chronic inflammatory reaction in the terminal bronchioles and alveoli of the lung with considerable fibrosis leading to distortion and eventual obliteration of the alveoli. There is also an association between asbestosis and the development of bronchogenic cancer² and Wagner *et al.*³ have noted the occurrence of diffuse pleural and peritoneal mesotheliomas in individuals occupationally exposed to asbestos dust and in experimental animals following intrapleural injection of asbestos.

It is accepted that the initial event after inhalation of toxic particles such as asbestos is their attachment to and often phagocytosis by mononuclear phagocytes (MP) in

the pulmonary alveoli⁴. As we consider that MP play a central role in the generation of chronic inflammatory lesions⁵, we have analysed the interaction of this type of cell with asbestos *in vitro*. Previous studies have illustrated the phagocytosis of asbestos by MP (refs 4 and 6) while biochemical evidence of the cytotoxic effects of relatively high doses of asbestos on MP has been provided by Miller and Harington⁷. We now show that asbestos, in common with several other substances such as dental plaque⁸, immune complexes⁹, streptococcal cell walls^{10,11} and carageenan¹²—all of which cause chronic inflammation *in vivo*—induces a rapid, massive and selective release of lysosomal enzymes from MP. The release occurs in the absence of any detectable cell death and is accompanied by considerable increases in cellular levels of non-lysosomal enzymes.

Our studies have been carried out with cultures of mouse peritoneal MP which are derived from the same bone marrow precursors as the majority of alveolar macrophages^{13,14} and are obtainable in sufficient quantities for detailed biochemical investigation. MP exposed to asbestos (UICC chrysotile A, 1-50 $\mu\text{g ml}^{-1}$) show characteristic morphological changes. The cells phagocytose asbestos and an increase in the number of cytoplasmic vacuoles, probably secondary lysosomes, is seen. At higher doses (25-50 $\mu\text{g ml}^{-1}$) the cells tend to form clusters and some rounding is seen although the majority of cells retain the cytoplasmic extensions characteristic of normal MP.

Measurement of the total level of lysosomal enzymes in MP cultures after exposure to asbestos for 24 h shows that in general there are no significant changes (Table 1). There is, however, a marked redistribution of lysosomal enzyme activity between cells and culture medium (Table 1), significant increases in the proportions of enzymes in the culture medium being seen with concentrations of asbestos as low as 1 $\mu\text{g ml}^{-1}$. This release is directly proportional to the concentration up to 50 $\mu\text{g ml}^{-1}$. The absolute amount of enzyme activity in the culture medium also increases in a dose-dependent fashion.

The selective nature of the lysosomal enzyme release is illustrated in Fig. 1. Asbestos at concentrations up to 50 $\mu\text{g ml}^{-1}$ causes no significant increase in the amount of

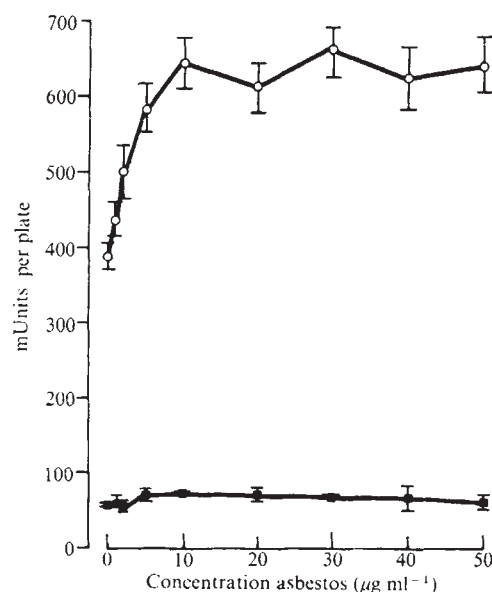


Fig. 1 Lactate dehydrogenase levels in MP culture exposed to increasing concentrations of asbestos for 24 h. ○, Activity in cells; ■, Activity in the culture medium. Each value represents the mean $\pm$ standard deviation of four observations. Experimental conditions were as described in Table 1. Lactate dehydrogenase was determined on a Technicon Autoanalyser by the method of Morgenstern *et al.*¹⁸.

Table 1 Lysosomal enzyme levels and distribution between cells and culture medium in MP cultures exposed to various concentrations of asbestos for 24 h

Asbestos concentration ($\mu\text{g ml}^{-1}$)	N-Acetyl- β -D-glucosaminidase nmol product per plate h^{-1}		β -Glucuronidase nmol product per plate h^{-1}		β -Galactosidase nmol product per plate h^{-1}	
	Total	% in medium	Total	% in medium	Total	% in medium
0	3,328 $\pm$ 228	13.9 $\pm$ 0.4	161.8 $\pm$ 4.3	18.2 $\pm$ 2.6	216.0 $\pm$ 17.3	11.3 $\pm$ 0.5
1	3,518 $\pm$ 170	20.2 $\pm$ 0.7†	171.1 $\pm$ 7.4	25.0 $\pm$ 4.1	210.7 $\pm$ 13.8	15.1 $\pm$ 0.9†
2	3,934 $\pm$ 387	20.1 $\pm$ 0.8†	183.1 $\pm$ 22.5	23.2 $\pm$ 2.6	221.7 $\pm$ 19.3	15.1 $\pm$ 0.5†
5	3,782 $\pm$ 294	28.5 $\pm$ 1.7†	167.9 $\pm$ 5.8	33.3 $\pm$ 2.7†	207.0 $\pm$ 12.8	21.6 $\pm$ 1.1†
10	4,150 $\pm$ 282*	30.8 $\pm$ 1.6†	203.4 $\pm$ 10.8†	38.8 $\pm$ 2.7†	224.9 $\pm$ 14.8	27.4 $\pm$ 1.3†
20	3,550 $\pm$ 210	43.5 $\pm$ 3.4†	180.5 $\pm$ 12.6	52.3 $\pm$ 4.4†	206.8 $\pm$ 11.3	44.5 $\pm$ 5.3†
40	3,089 $\pm$ 249	53.4 $\pm$ 6.3†	167.0 $\pm$ 11.3	67.1 $\pm$ 4.9†	180.7 $\pm$ 14.8	60.9 $\pm$ 2.0†
50	3,615 $\pm$ 276	61.3 $\pm$ 5.9†	183.0 $\pm$ 19.2	77.5 $\pm$ 5.0†	206.0 $\pm$ 8.3	69.8 $\pm$ 5.2†

Peritoneal exudates were collected from outbred Swiss mice of either sex 3 d after intraperitoneal injection of 2 ml of proteose-peptone cell suspensions (5 ml) adjusted to a count of $0.8 \times 10^6 \text{ ml}^{-1}$ were plated in 48-mm Petri dishes (Nunc, Jobling Laboratories Division, Stone, Staffs, UK). After culture for approximately 1 h at 37°C in a 5% CO_2 in air atmosphere the medium containing non-adherent cells was removed and the remaining cell sheet washed four times with 4 ml aliquots of phosphate-buffered saline. The cells were then cultured overnight in M199 containing 10% (heat-inactivated at 56°C for 30 min) swine serum (Bio-cult Laboratories Ltd., Glasgow, Scotland), Chrysotile asbestos (UICC standard) was sonicated (MSE ultrasonic disintegrator) in serum-free M199 for 2 min. M199 and swine serum was then added to give the desired concentration of asbestos and a final swine serum concentration of 10%. Asbestos was preincubated in medium overnight. Before adding asbestos the cultures were washed twice with phosphate-buffered saline. After exposure to asbestos for 24 h the cultures were assayed for various enzymes. After removal of the culture medium the cells were removed in 0.1% TX-100 in 0.9% w/v sodium chloride containing 0.1% bovine serum albumin. The samples were assayed for N-acetyl- β -D-glucosaminidase¹⁵, β -glucuronidase¹⁶ and β -galactosidase¹⁷ activity by spectrophotometry on a Gilford N-300 spectrophotometer. Each value represents the mean $\pm$ standard deviation of four observations.

* $P < 0.05$.

† $P < 0.01$.

lactate dehydrogenase detected in the culture medium. Moreover, even small amounts of asbestos induce highly significant increases in cellular levels of this enzyme (Fig. 1, $P > 0.01$ at $2 \mu\text{g ml}^{-1}$ asbestos). This increase continues in a dose-dependent manner up to $10 \mu\text{g ml}^{-1}$ asbestos, above which lactate dehydrogenase activity remains at a constant elevated level. Similar increases were observed with another non-lysosomal, smooth membrane associated enzyme, leucine-2-naphthylamidase.

The time dependence of the selective release of lysosomal enzymes caused by asbestos is shown in Fig. 2. MP cultures exposed to asbestos ($50 \mu\text{g ml}^{-1}$) show no change compared to control cultures, in the distribution of a representative lysosomal enzyme, β -galactosidase, between cells and culture medium for the first 3 h. By 4.5 h, however, a highly significant increase ($P < 0.01$) in the amount of β -galactosidase in the medium is seen. Subsequently a rapid rise in the release of enzyme into the culture medium occurs so that by 24 h approximately 70% of the total enzyme activity is found in the culture medium. As in the other experiments described in Table 1 and Fig. 1, this release occurs with no detectable loss of cellular lactate dehydrogenase into the culture medium and significant increases in cellular levels of this enzyme at 24 h. The delay in release is most probably due to the coating of ingested asbestos fibres with plasma proteins. These proteins are removed within secondary lysosomes by proteolytic digestion thereby exposing the membrane reactive sites, probably magnesium ions¹⁹, on the asbestos fibres. It is thought that the magnesium groups of asbestos interact with membrane sialoglycoproteins²⁰ and it is possible that such an interaction leads to the formation of protein clusters in membranes, a phenomenon observed during exocytosis in other systems²¹. The toxic effect of silica on MP is well established and is attributed to the formation of hydrogen bonds between hydroxyl groups of silicic acid and membrane phospholipids. Silica does not cause any selective release of lysosomal enzymes (P. D., A. C. A., J. A., A. B. and S. W., unpublished); the appearance of acid hydrolases in the culture medium being accompanied always by lactate dehydrogenase. These observations support the concept that clustering of membrane proteins is an essential prerequisite to membrane fusion such as occurs during exocytosis²².

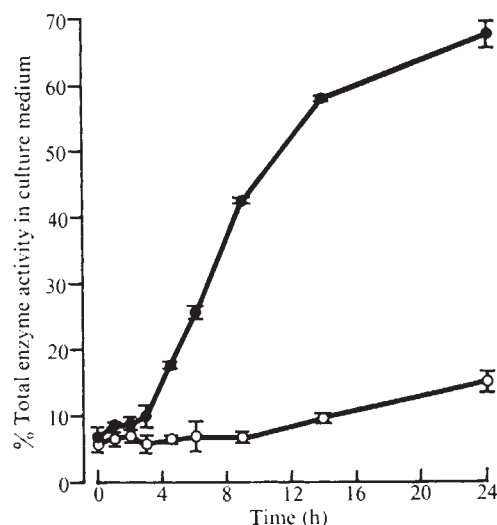


Fig. 2 The time dependence of β -galactosidase release from MP induced by asbestos. MP cultured in the presence (●) and absence (○) of asbestos ($50 \mu\text{g ml}^{-1}$) were removed at various times for enzyme assay. Each value represents the mean $\pm$ standard deviation of four observations. Experimental conditions as described in Table 1.

The sensitivity of MP cultured *in vitro* to toxic particulates such as asbestos and silica provides a simple and sensitive way of screening various particulate substances for possible toxic effects. It is insufficient to monitor cell viability, however. We have demonstrated that asbestos can release a large proportion of cellular content of MP acid hydrolases without detectable cell death. It is essential to determine the distribution of lysosomal enzymes between cells and culture medium. This type of estimation is particularly relevant since several agents which cause chronic inflammation *in vivo* induce selective release of lysosomal enzymes from MP *in vitro*⁸⁻¹²; accordingly we have found (M. Dym, P. D., and A. C. A., unpublished) that asbestos produces an intense granulomatous reaction after intramuscular injection in mice.

After intrapleural injection of asbestos, a marked mononuclear reaction is also observed; this subsides shortly before mesotheliomas appears²³, raising the possibility that the mononuclear phagocytes in the granuloma prevent the multiplication of malignant cells²⁴ so delaying the appearance of the tumour.

The results reported here have relevance in at least four different fields of study. First, the experimental system used provides a simple and sensitive *in vitro* test for the possible cytotoxicity of particulate substances. Second, our observations on the selective release of lysosomal enzymes from MP by asbestos provide further support for the possibility that this phenomenon may be responsible for certain aspects of tissue damage seen in chronic inflammation^{5,12}. Third, the use of biologically active substances of defined chemical structure such as asbestos may provide a useful research tool for determining the nature of membrane constituents involved in changes which lead to membrane fusion²². Fourth, some granuloma-inducing material other than asbestos might be administered by intrapleural injection to persons who have in the course of their occupations been exposed to asbestos so as to maintain a local granulomatous reaction which might prevent the appearance of mesotheliomas and bronchogenic cancer.

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Modulation of corticotrophin-releasing factor release from hypothalamic synaptosomes

NERVE endings (synaptosomes) isolated from the mammalian hypothalamus contain the hypophysiotrophic hormones which regulate adeno-hypophysial function. Edvardson *et al.* have shown¹ that release of these neurosecretory substances can be elicited from incubated hypothalamic synaptosomes by electrical or potassium stimulation and the release mechanism has been shown² to be calcium dependent. We report here that steroid hormones administered both *in vivo* and *in vitro* and neurotransmitter substances given *in vitro* modulate the release of corticotrophin-releasing factor (CRF) from synaptosomes prepared from rat and sheep hypothalamus.

Adult rats were given corticosterone acetate in drinking water (20 $\mu\text{g ml}^{-1}$) for a period of 20 h (average dose, 300 μg per rat) before being killed. Synaptosomes were prepared from the hypothalami by a modified³ version of the method of Gray and Whittaker⁴ and suspended in Krebs-bicarbonate medium containing 10 mM glucose. Suspensions of synaptosomes were incubated and stimulated electrically in Warburg respirometer vessels fitted with concentric gold ring electrodes as described previously¹. After incubation the preparations were centrifuged and the supernatant medium was tested for CRF activity. Paired halves of rat adeno-hypophyses were incubated in 1 ml of the medium and adrenocorticotrophic hormone (ACTH) release was measured by radioimmunoassay⁵. The antiserum used was raised against the species common, steroidogenic $\alpha 1$ -24ACTH and does not show significant cross reaction with pituitary melanocyte-stimulating hormone.

Medium from stimulated synaptosomes prepared from untreated rats gave a 61% increase in ACTH release compared with medium from unstimulated preparations (Table 1). Pretreatment with corticosterone completely abolished this response. This was due to absence of CRF rather than to an inhibitory effect of the medium on the pituitary response, since a test stimulus of 40 mU vasopressin added with the same synaptosome medium produced a 144% increase in ACTH release.

Table 1 The effect of *in vivo* pretreatment with corticosterone on the release of CRF by synaptosomes from rat hypothalamus

Preparation	No. of experiments	ACTH released (ng ml ⁻¹)		
		Un-stimulated	Electrically stimulated	% Increase
Untreated	5	28.1 $\pm$ 4.3	45.9 $\pm$ 8.5*	61
Corticosterone pretreatment <i>in vivo</i>	6	20.0 $\pm$ 2.7	19.8 $\pm$ 2.4	0
Corticosterone pretreatment <i>in vivo</i> + vasopressin test <i>in vitro</i>	6	18.6 $\pm$ 2.0	45.5 $\pm$ 4.2	144

Values were determined by radioimmunoassay using $\alpha 1$ -24 antibodies and represent ACTH released from paired halves of rat adeno-hypophyses during a 45 min incubation period at 37° C with synaptosome supernatant. Corticosterone was administered in the drinking water for 20 h before preparation of synaptosomes (average dose 300 μg per rat). A dose of 40 mU ADH was added to medium from stimulated synaptosomes prepared from corticosterone pretreated rats to show that the response of the test pituitary was normal.

*Wilcoxon test, $P < 0.05$.

Table 2 The effect of steroids on the release of CRF produced by electrical stimulation of synaptosomes from the sheep hypothalamus

Hormone	Concentration (M)	No. of experiments	ACTH released ng ml ⁻¹		% Increase
			Unstimulated	Stimulated	
None	—	10	28.5 ± 4.0	59.3 ± 7.5*	108
Corticosterone	7.5 × 10 ⁻⁷	6	31.3 ± 4.2	36.3 ± 5.9	16
Cortisol	6.0 × 10 ⁻⁷	5	49.2 ± 13.3	48.4 ± 13.1	0
Cortisol	6.0 × 10 ⁻⁸	6	62.3 ± 8.2	62.9 ± 12.0	10
Cortisol	6.0 × 10 ⁻⁹	5	49.1 ± 4.8	65.4 ± 8.1	33
Deoxycorticosterone	8.0 × 10 ⁻⁷	6	34.7 ± 4.0	58.9 ± 8.7†	70
Deoxycortisol (Substance S)	8.5 × 10 ⁻⁷	5	43.4 ± 7.5	88.2 ± 18.0‡	101
Progesterone	9.5 × 10 ⁻⁷	3	42.1 ± 6.4	68.2 ± 13.1	62
Oestradiol	1.1 × 10 ⁻⁶	3	37.6 ± 6.2	57.0 ± 8.5	52
Deoxycortisol + cortisol	8.0 × 10 ⁻⁷	3	21.3 ± 4.3	42.2 ± 6.0	99

Values were determined by radioimmunoassay, as explained in Table 1 (means ± s.e.m.). Steroids were present during a 35 min incubation of synaptosomes and electrical stimulation was for 25 min. Wilcoxon test, * $P < 0.005$; † $P < 0.025$; ‡ $P < 0.05$.

A direct action of corticosteroids on the nerve ending was shown in further experiments with synaptosomes from the hypothalami of sheep killed by exsanguination. This species was chosen because of the relatively large amount of hypothalamic tissue available compared with the rat, and the synaptosomes were prepared as described previously. Steroid hormones were added to synaptosome suspensions which were incubated for 15 min without stimulation, followed by 25 min electrical stimulation. Hormones were added to the controls after stimulation to eliminate any differences due to inhibition of ACTH release by steroid in the medium. Where solubility of the steroid hormones in medium did not permit direct addition to the synaptosome suspension, they were first dissolved in absolute ethanol, placed in the Warburg vessels and evaporated to dryness before the synaptosomes were added.

Medium from synaptosomes stimulated in the absence of steroids produced a 108% increase in ACTH secretion compared with medium from the unstimulated preparation (Table 2). This stimulation of ACTH release was reduced to 16% by the presence of corticosterone at 7.5×10^{-7} M and completely abolished by cortisol at the same concentration. These concentrations of steroid are comparable with amounts present in the circulation but may not be physiological because of the absence of the plasma proteins to which they are normally bound. Cortisol, the chief glucocorticoid in the sheep, did however, block CRF release

at 6.0×10^{-8} M and even at 6.0×10^{-9} M produced a substantial inhibition. The small size of this effective dose, which approximates to the levels of unbound cortisol in blood measured during stress, and its action within a relatively short time suggest that the neurosecretory nerve terminal may be an important site of action in the fast feedback control of ACTH release by corticosteroids which has been described recently⁶.

The results in Table 2 show that there is some variation in the control values for ACTH secretion. The assay procedure involved the use of paired halves of adenohypophyses for simultaneous testing of stimulated and unstimulated preparations, however, and a valid comparison can be made between these two groups using the Wilcoxon matched-pairs signed-ranks test.

Results obtained with other steroids, suggest that the inhibitory effect of CRF release may be of a relatively specific nature. Progesterone and 17β -oestradiol at a higher molar concentration than cortisol produced a partial inhibition of CRF release. It has been shown⁷, however, that a hormone such as 17α OR-progesterone, which has no glucocorticoid activity, may suppress the stress-induced release of ACTH in rats. The failure of deoxycortisol, the immediate precursor of cortisol in adrenal steroidogenesis, to inhibit CRF release is of interest. Preliminary experiments have shown that after preincubation with deoxycortisol, cortisol is no longer effective in inhibiting the electrically

Table 3 The effect of neurotransmitters on the release of CRF by synaptosomes isolated from the sheep hypothalamus

Test	Concentration (M)	No. of experiments	ACTH release (ng ml ⁻¹)		% Increase
			Control	Experimental	
Electrical stimulation (E)	—	10	28.5 ± 4.0	59.3 ± 7.5*	108
Acetylcholine (E)	4.4 × 10 ⁻¹¹	5	45.3 ± 7.8	76.9 ± 13.3	69
Acetylcholine (E)	2.2 × 10 ⁻¹⁰	5	44.7 ± 7.4	102.2 ± 22.0†	142
Acetylcholine (E)	4.4 × 10 ⁻⁹	6	38.4 ± 6.1	123.7 ± 15.2*	223
Atropine (C, E) + acetylcholine (E)	2.9 × 10 ⁻⁹	6	34.7 ± 6.7	41.8 ± 7.3	21
Dopamine (E)	6.5 × 10 ⁻⁸	4	25.9 ± 5.4	25.7 ± 3.5	0
Dopamine (C, E) + acetylcholine (E)	6.5 × 10 ⁻⁸	6	49.9 ± 3.8	62.6 ± 6.4	25
Dopamine (C, E) + electrical stimulation (E)	2.2 × 10 ⁻¹⁰	6	48.3 ± 5.6	52.8 ± 2.3	9
Noradrenaline (C, E) + acetylcholine (E)	6.5 × 10 ⁻⁸	3	29.6 ± 1.7	69.6 ± 19.4	135
5-Hydroxytryptamine (C, E) + acetylcholine (E)	2.2 × 10 ⁻¹⁰	3	32.9 ± 5.0	79.7 ± 6.4	142

Values were determined by radioimmunoassay as explained in Table 1. Where a single neurotransmitter is indicated as the test, this was present in the experimental flask (E) only. Where the effects of neurotransmitters or atropine on the release of CRF evoked by electrical stimulation or acetylcholine were investigated the former agents were present in both control (C) and experimental (E) flasks. Neurotransmitters were present during the 35 min incubation of synaptosomes and electrical stimulation, when used, was for the last 25 min of this period. CRF release in the presence of noradrenaline and 5-hydroxytryptamine alone did not differ significantly from control unstimulated values. None of the transmitters at the concentration used had a direct effect on the pituitary response to medium from stimulated synaptosomes. Wilcoxon test, * $P < 0.005$; † $P < 0.025$.

stimulated release of CRF and the results suggest that competitive inhibition may occur for binding sites which exist at the level of the nerve terminal. These results are in good agreement with those⁸ obtained from *in vivo* studies on the interactions of corticosteroids involved in feedback control of ACTH secretion. The findings are of particular interest in that they suggest a physiological action of a steroid hormone which does not involve a nuclear site.

Further evidence that the neurosecretory terminal is an important target for substances involved in the modulation of the hypophysiotrophic activity of the hypothalamus was obtained when neurotransmitter substances were added to suspensions of synaptosomes prepared from the sheep hypothalamus (Table 3). Acetylcholine in concentrations varying from 4.4×10^{-9} to 10^{-11} M stimulated the release of CRF in a dose-dependent manner. The response was not enhanced by eserine (results not shown) but it was blocked in the presence of atropine (2.9×10^{-9} M) suggesting the existence of specific cholinergic receptors on the CRF containing nerve terminal. Dopamine (6.5×10^{-8} M) blocked the release of CRF produced by acetylcholine or electrical stimulation. Noradrenaline at similar concentrations caused only a partial inhibition of CRF release and 5-hydroxytryptamine was without effect. The results are compatible with findings obtained in the whole animal⁹⁻¹¹ and with the isolated hypophysiotrophic hypothalamus¹² *in vitro* and are consistent with an excitatory cholinergic and inhibitory dopaminergic modulation of CRF secretion. The effects obtained with acetylcholine and dopamine on synaptosomes probably require that axo-axonal endings be present in the median eminence if the results obtained *in vitro* are to be considered as having any relation to what may be happening *in vivo*. While it seems likely that such dopaminergic terminals exist¹³ there is at present no anatomical evidence for a cholinergic synapse with neurosecretory neurones in the median eminence. In view of the specificity and sensitivity of the effect obtained with acetylcholine, however, we suggest that humorally mediated neurotransmitter may be active at the nerve ending *in vivo*. The findings obtained with steroids and neurotransmitters emphasise the potential importance of control mechanisms which may operate at the level of the neurosecretory nerve terminal in neuroendocrine integration.

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Seasonal variation in sensitivity of guinea pig tissues to agonists

WE have observed a marked and consistent seasonal variation in the sensitivity of guinea pig tissue to acetylcholine (ACh). Ileum taken from guinea pigs during the summer months (May to September) were much less sensitive to the stimulant effect of ACh than those removed during the winter (November to February). Dawson, Hemsworth and Stockham¹ also noted that the response of the ileum to spasmogens varied widely at different times of the year, but they gave no specific details.

The purpose of these preliminary investigations was to see whether two other smooth muscle stimulants, 5-hydroxytryptamine (5-HT) and potassium chloride (KCl), which act in different ways from that of ACh, showed a similar change in sensitivity. The stimulant action of 5-HT is mediated mainly through stimulation of nervous elements causing the release of endogenous ACh, but it also has some direct action on the longitudinal muscle². KCl is believed to use a different source of calcium from that of ACh when causing contraction of smooth muscle³.

To see whether heart muscle also showed different sensitivity in the summer and winter we studied the positive chronotropic effect of isoprenaline on isolated guinea pig atria.

Male guinea pigs weighing 250-500 g were used. They were housed in uniform conditions, three per cage in rooms maintained between 22° C and 25° C throughout the year. The animals were fed on a standard diet of food pellets, (Amber Ltd) mixture number 19350. The pellets were coated with ascorbic acid, 1 mg g⁻¹. The average daily intake of ascorbic acid was 50-60 mg per guinea pig.

Guinea pigs were killed by cervical dislocation and a 4 cm piece of ileum was removed about 20 cm from the ileocaecal junction. The ileum was suspended in a 5 ml isolated organ bath containing Krebs-Henseleit solution aerated with a mixture of 95% O₂ and 5% CO₂, at a tension of 1.5-2 g, and maintained at a temperature of 36°-37° C. Responses of the ileum to the spasmogens were recorded isotonicly with a Satham strain gauge on a multichannel polygraph.

Isolated guinea pig atria were set up under the same conditions, but the temperature was reduced to 30°-31° C. Dose-response curves were established to ACh, 5-HT and KCl on guinea pig ileum by adding the drug at 2 min intervals; the bath fluid was changed after each dose. On isolated atria cumulative dose-response curves for the increase in chronotropic effect were obtained for isoprenaline by adding increasing amounts of the amine to the bath and washing out only after a maximum increase in rate was achieved, as described by Weinstock *et al.*⁴.

The concentration of drug in each case required to give 50% of the maximum response (ED₅₀) was determined. The winter and summer values for the ED₅₀ for ACh, 5-HT, KCl and isoprenaline are shown in Table 1.

The change from summer to winter brought about a general increase in sensitivity (lower ED₅₀) of both cardiac and smooth muscle to adrenergic and cholinergic agents. Moreover, the difference in responsiveness of the ileum in the winter from that in the summer was much greater for ACh and 5-HT than for KCl. Such a change in response of smooth muscle to agonists is reminiscent of the nonspecific increases in sensitivity seen after de-

Table 1 Seasonal responses of isolated guinea pig tissues to various agonists

Tissues	Drug	Season	n	ED ₅₀ mean $\pm$ s.e. (ng ml ⁻¹)	Level significant (between winter + summer values)
Ileum	Acetylcholine	Nov.-Feb. 1971-72	6	2.7 $\pm$ 0.8	0.001
Ileum	Acetylcholine	May-Sept. 1972	13	40.0 $\pm$ 4.7	
Ileum	Acetylcholine	Nov.-Feb. 1972-73	9	6.5 $\pm$ 0.6	0.002
Ileum	Acetylcholine	May-Sept. 1973	15	44.6 $\pm$ 10.2	
Ileum	Acetylcholine	Nov.-Feb. 1973-74	12	6.0 $\pm$ 1.1	
Ileum	5-Hydroxytryptamine	May-Sept. 1973	6	1,250 $\pm$ 25	0.001
Ileum	5-Hydroxytryptamine	Nov.-Feb. 1973-74	8	16.9 $\pm$ 3.3	
Ileum	Potassium chloride	May-Sept. 1973	6	0.79 $\pm$ 0.17 mg ml ⁻¹	0.05
Ileum	Potassium chloride	Nov.-Feb. 1973-74	5	0.31 $\pm$ 0.06 mg ml ⁻¹	
Atria	Isoprenaline	May-Sept. 1973	8	3.60 $\pm$ 0.68	0.001
Atria	Isoprenaline	Nov.-Feb. 1973-74	5	0.184 $\pm$ 0.004	

centralisation or reserpine treatment; Westfall⁵ found that the increase in response of the guinea pig vas deferens to noradrenaline and methylfurmethide (a cholinergic stimulant) was greater than that to KCl.

Dawson, Hemsworth and Stockham¹ found that a daily intake of 50 mg of ascorbic acid in the diet of guinea pigs resulted in an increase in sensitivity of the ileum to spasmogens. As in our study the animals received this amount of ascorbic acid daily throughout the year, however, we have to seek a different explanation for the seasonal supersensitivity.

All our experiments were carried out in solutions of identical calcium and magnesium ion concentration, which suggests that the difference in seasonal response, like that after decentralisation, may be related to a change in some property of the membrane binding site for calcium. It does not seem to be due to a change in blood level of thyroxine, since it has been shown that this hormone increases sensitivity of the guinea pig heart to catecholamines but decreases the responsiveness of the ileum to ACh (ref. 6). It may, however, be related to cyclic enzymatic or other endocrinological changes. Johansson and Santuria⁷ reported that the guinea pig heart has a higher noradrenaline level in the summer than in the winter, which could explain the reduced sensitivity to exogenous isoprenaline. They also found that serum calcium and potassium were higher in the winter than in the summer.

We would be interested to hear whether a similar change in sensitivity of guinea pig tissues occurs in other laboratories, particularly in those in which winter falls in June-July and whether tissues from other laboratory animals are similarly affected.

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Morphological evidence for an experimentally induced synaptic field

WHEN fibres in the central nervous system (CNS) of invertebrates and lower vertebrates are interrupted, regenerating axons grow beyond the lesion and form new synapses on denervated neurones. In the leech CNS, however, we have seen synapses formed by the regenerating fibres at the site of the lesion. The formation of synapses in regions where they are normally absent raises important questions about the manner in which axons grow to their targets and form orderly connections.

The CNS of the leech consists of segmental ganglia and longitudinal connectives. The ganglia have a cell rind formed by nerve cell perikarya and a central fibrous neuropile containing most of the synapses. The neuropiles are joined together by the connectives, which include axons of interneurons and sensory nerve cells. The axons (0.1-9 μ m in diameter) run caudally and rostrally and form three longitudinal bundles of fibres associated with the cytoplasm of two giant glial cells. The cells are found in the two lateral bundles and their nuclei lie between successive ganglia¹⁻⁴. Many of the nerve fibres are enclosed individually within invaginations of the giant glial cell processes, and synaptic junctions are rare in the nerve bundles.

A lesion in a connective interrupts the neural pathways linking the ganglia^{5,6} and damages the giant glial cells. Transected axons soon grow across the lesion, however, and form a neuroma enclosing a different kind of glial cytoplasm. Since the animals soon swim normally again, functional connections between the isolated parts of the nerve cord must be re-established during regeneration. A plausible explanation for this would be that regenerating fibres grow along the injured connective, and synaptic connections are restored at the ganglionic neuropile. We have found, however, that regenerating axons build up a new neuropile at the site of the lesion. This neuropile includes a synaptic field very similar to that in the ganglionic neuropile.

Twelve leeches of the species *Macrobdella decora* were anaesthetised with 8% ethanol and lesions were placed at the centre or at one end of the connective joining either ganglia 3 and 4 or 4 and 5. Each lesion was a discrete or extensive crush that often also involved the immediate walls of the haemocoelom which encloses the entire nerve cord. Although lesions resulted in abnormal swimming, normal behaviour resumed within 4 weeks. Animals survived in tanks with deionised water at 20-25° C and were killed 4, 19, 30, 55, 86 and 111 d after the operation. We started examining 4-d-old neuromas because at this stage axons growing from both stumps of the severed connective met across the lesion.

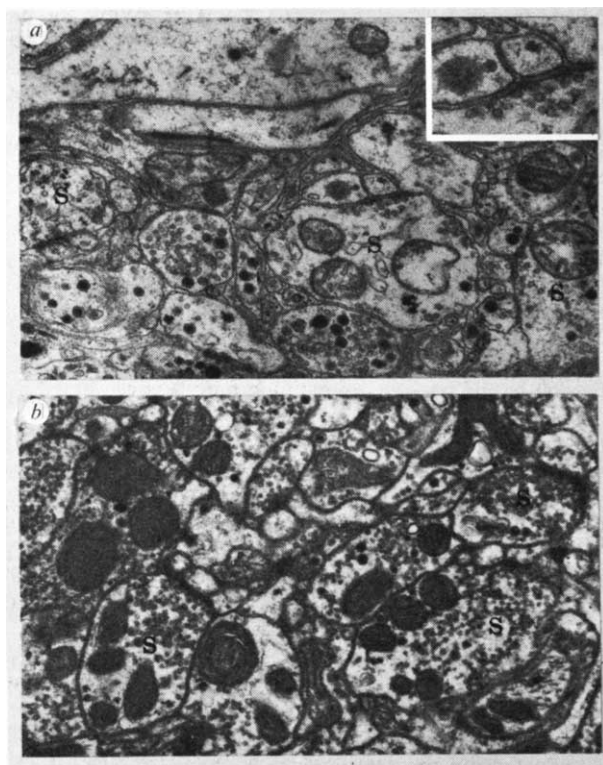


Fig. 1 Low magnification electron micrographs of 111-d-old neuroma (a) and normal ganglionic neuropile (b) that demonstrate the striking structural similarity between both kinds of neuropiles. Divergent types of synaptic clusters (S) are shown. Inset shows the specialised junction formed between the postsynaptic fibres. (a, $\times 16,280$; inset, $\times 29,304$; b, $\times 14,080$).

Light microscopy showed that the lesions damaged part of the haemocoelom walls and underlying nerve connective, leaving cell debris which was ultimately removed by macrophages. Meanwhile many axons, growing from both ends of the lesion, advanced towards each other across the haemocoelom walls and formed a neuroma. Serial sections revealed the absence of giant glial cell cytoplasm across the nerve tissue, which consisted of many small and a few large fascicles near the twisted haemocoelomic canal.

Electron microscopy of neuromas of different ages confirmed the absence of giant glial cell cytoplasm, but revealed that of many small glial cells, not found in the normal connective. In very young neuromas (4 d), most immigrating glial cells appeared to be confined to the surface of regenerating tissue. In older neuromas, however, such cells were widespread.

The neuroma gradually transformed into a neuropile-type structure (Fig. 1a), with the nerve processes resembling those of the ganglionic neuropile (Fig. 1b). Thus, many neural profiles lacked a glial lining, or it extended only partly around their perimeter, leaving more direct surface contact between the nerve fibres than in the normal connective. Axons frequently formed axo-axonic synapses and contained many vesicular profiles of both granular (600–1,200 Å in diameter) and agranular (400 Å in diameter) types. The latter were particularly numerous in the pre-synaptic axons, mostly lying adjacent to the synaptic membrane. Here there was also electron dense material that was usually thicker than that associated with the postsynaptic membrane. The synaptic cleft measured approximately 180 Å in diameter (Fig. 2).

In both ganglionic and experimentally induced neuropiles, axo-axonic synapses were mostly in clusters, usually including one presynaptic and several postsynaptic fibres. Thus, the key feature of organisation of the synaptic clusters was

divergence (Figs 1 and 2). When clusters included more than one afferent fibre, however, there was sometimes convergence. The extracellular space between postsynaptic fibres often narrowed and specialised neural junctions were formed (Fig. 1a).

Synaptic clusters were present in 4-d-old neuromas (Fig. 2a) and their numbers increased during the first weeks after the operation. The fact that a synaptic field was still present in the oldest neuromas we examined (111 d) is indicative of its nontransient character (Figs 1a and 2b).

What causes the regenerating nerve fibres of the leech to form an extraganglionic synaptic field at the site of injury? First, it seems likely that local conditions at the lesion favour direct interaction between growing axons. One such condition is the early absence of glial cell barriers throughout the regenerating tissue. This is associated with both the failure of the giant glial cells to repair damaged parts and the delayed invasion of the neuroma by glial cells. The competence of axons to synapse is probably associated with the early availability of new synaptic sites at the axon surface. These sites may arise, at least in part, as a consequence of collateral axon sprouting, which can be induced by, for example, partial denervation and nerve injury⁷⁻¹². Some collateral axonal sprouts may come from undamaged nerve fibres. However, it is reasonable to suspect that most would originate from regenerating fibres growing across the lesion site. This view is supported by the fact that transected axons in the nerve connectives of the leech sprouted vigorously compared with those in the CNS of higher vertebrates^{12,13}. Finally, there is evidence that axonal sprouts can form new synaptic junctions when they meet the receptive surface of a neurone or muscle cell^{12,14}.

What type of neurones form synapses at the neuroma? Many neurones of neighbouring ganglia are linked synaptically⁶ and many of them recover their junctions after partial severance of a nerve connective^{15,17}. Thus many neurones may re-establish at least some of their specific connections at the neuroma. This implies that many of the synapses would

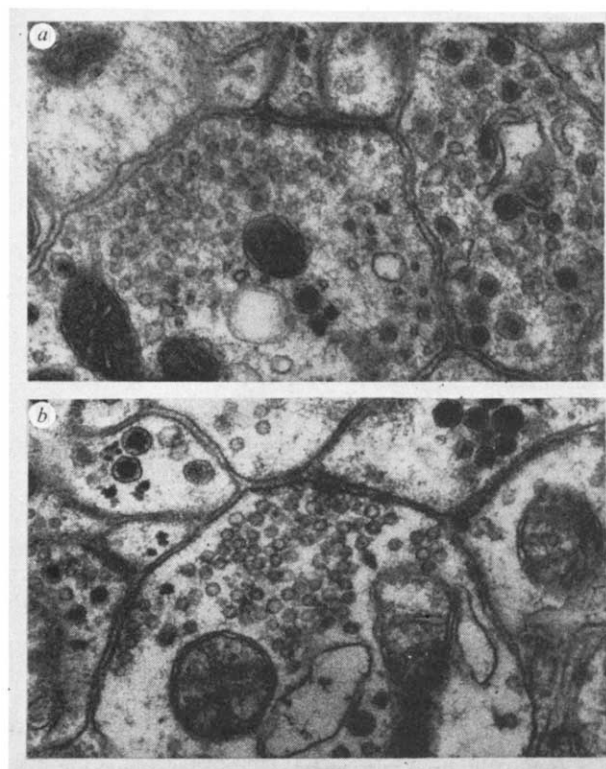


Fig. 2 High magnification electron micrographs of synaptic clusters in 4-d-old (a) and 111-d-old neuromas (b). In both cases, the cluster includes a single presynaptic fibre ($\times 40,480$).

be formed between the central processes of neurones growing from opposite stumps of the severed connective. The fact that the conduction of action potentials is regained across the neuroma¹⁵ and that some regenerating fibres grow beyond this structure (our unpublished work) suggests that axons also restore their specific connections at the ganglionic neuropile. Hence, a given pair of neurones of successive ganglia might renew their interconnections completely either at one or both neuropiles. Alternatively, many of the synapses found at the neuroma may be duplicates of those formed by the same regenerating fibres at the level of the ganglia. Thus, there would be a factor of redundancy in regenerated synapses. This might explain some consistent differences between regenerated and normal synapses with regard to the balance of excitation and inhibition¹⁷.

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Effect of prostaglandins E₁, E₂ and F_{2α} on human blood coagulation

As prostaglandins are being used to induce labour and abortion in some women, it is necessary to know the effect of these compounds on the blood coagulation system. We have investigated this problem using plasma from normal women and prostaglandins E₁, E₂ and F_{2α} (Upjohn).

The prostaglandins, dissolved in absolute ethanol, were added to samples of the plasma to give a final concentration of 1, 10 and 100 ng ml⁻¹. Each 0.1 ml of plasma contained the equivalent of 1.0 ng of ethanol, which was added to similar samples of plasma during control runs of coagulation tests. After the addition of prostaglandins, aliquots of plasma were incubated for 1 h at 37° C to determine whether there was any

conversion to prostaglandins A₁ or B₁^{1,2}. While preparing 'platelet-rich plasma' we minimised the release of prostaglandin from the platelets. We used slightly modified versions of established methods to study blood coagulation in ten samples of plasma from each of ten subjects. To 0.2 ml of control and prostaglandin-treated plasma we added one of the following: thromboplastin, to measure prothrombin time³; cephaloplastin (Dade, Miami) to measure partial thromboplastin time⁴, and thrombin (0.7 U per 0.1 ml, Fibrinex, Ortho) to measure thrombin clotting time. Percentage clot retraction was estimated by addition of 0.1 ml of cephaloplastin to 0.8 ml of platelet-rich plasma, followed by 0.1 ml of 0.1 M CaCl₂ in 1.0-ml graduated tubes into which were inserted applicator sticks. This mixture was incubated for 30 min at 37° C. After removal of the clot adhering to the stick, the remaining fluid was estimated as the percentage of the original 1.0 ml.

Plasma factors, V, VII, VIII, IX, X, XI and XII were determined by a modification of the test for prothrombin and partial thromboplastin time, using plasma from subjects deficient in the various factors as the vehicle for the clotting effect on control and prostaglandin-treated plasma. The plasma factor assays for the several prostaglandins *in vitro* were assessed at the 100 ng level only. Coagulation tests were also carried out on plasma from several pregnant women receiving prostaglandin F_{2α} or Pitocin for induction of labour, or Pitocin for abortion, and from women at the time of delivery (unpublished results of R. K. Laros).

None of the prostaglandins in concentrations of 1–100 ng ml⁻¹ affected the coagulation tests or the plasma factor assays. Similarly, none of the plasma from the *in vivo* study (unpublished results of R. K. Laros) yielded any significant deviation from normal values for prothrombin time, partial thromboplastin time, thrombin clotting time or percentage clot retraction. *In vivo* studies on patients receiving prostaglandin F_{2α} as a means of inducing abortion also independently supports this conclusion⁵. None of the plasma samples converted the added prostaglandins to A₁ or B₁, although this has been described elsewhere¹. Tables 1 and 2 summarise the *in vitro* findings.

Table 1 Effect of prostaglandins E₁, E₂ and F_{2α} on coagulation tests on human blood plasma

Prosta- glandin	ng	Mean* PT (S)	Mean† PTT (S)	Mean‡ TCT (S)	Mean§ clot retraction
E ₁	1	10.2	31.3	8.8	83.0
E ₁	10	10.1	30.3	8.8	80.0
E ₁	100	10.2	32.5	8.8	70.0
E ₂	1	10.1	32.0	8.3	80.0
E ₂	10	10.3	32.2	8.8	80.0
E ₂	100	10.3	32.5	8.8	85.0
F _{2α}	1	10.0	31.0	8.8	85.0
F _{2α}	10	10.2	32.0	8.8	75.0
F _{2α}	100	10.3	31.0	8.3	80.0
Mean Control		10.2	30.5	8.8	80.0

Clot retraction is expressed as percentage of control. PT, Thromboplastin time; PTT, partial thromboplastin time; TCT, thrombin clotting time.

* Range, 10–16 s.

† Range, 30–33.0 s.

‡ Range, 8.0–9.0 s.

§ Range, 70.0–90.0%.

Prostaglandins E₁ and E₂, apparently at physiological (1.0 ng ml⁻¹ plasma) as well as the pharmacological levels we used, do not affect the clotting mechanism other than by inhibiting platelet aggregation and adhesiveness on the part of prostaglandin E₁. Blood samples from patients obtained before infusion of prostaglandin F_{2α} and at delivery, when prostaglandin concentrations are maximum and immediately *post partum* were relatively unchanged, although significant coagulation abnormalities developed in patients being aborted with the aid of Pitocin (unpublished results of R. K. Laros).

Table 2 Effect of prostaglandins E₁, E₂ and F_{2α} on plasma coagulation factors tests

Prosta- glandin	ng	Mean factor V	Mean factor VII	Mean factor VIII	Mean factor IX	Mean factor X	Mean factor XI	Mean factor XII
E ₁	100	104	100	73.0	90.0	100	102.0	88.0
E ₂	100	101	147	117.0	87.0	113	83.0	92.0
F _{2α}	100	104	100	77.0	90.0	100	96.0	106.0
Control (1 ng ethanol)		100	100	100.0	100.0	100	100.0	100.0

Values are expressed as mean percentage of control.
Ranges for factors: V, 100–108; VII, 100–150; VIII, 70–110; IX, 90–100; X, 100–106; XI, 100–104; XII, 80–100.

Prostaglandins seem to affect haemostasis through the platelet. The ability of prostaglandins to interfere with platelet aggregation has been well established and may represent one of the major effects of prostaglandins on the clotting mechanism, although less well documented. Ferri *et al.*⁷ noted a shortening of the recalcified clotting time in citrated rat blood, suggesting the development of a hypercoagulable state. Kloeze⁸, on the other hand, also working with rat plasma could not alter the prothrombin time, but changed the thromboelastogram pattern of prostaglandin-containing blood, an effect probably related to platelet function. In contrast to our findings, Murer⁹, comparing different systems of clot retraction, noted an inhibitory effect of prostaglandin E₁ on diluted calcium-deficient plasma solutions clotted with thrombin. Addition of calcium reversed the inhibition, resulting in normal clot retraction. Suspensions of washed platelets where clots were formed by fibrin monomers and calcium also were inhibited by prostaglandin E₁ and were not improved by further addition of calcium⁹. Addition of calcium to the platelet-rich plasma alone in our system was unaffected by prostaglandins E₁, E₂ and F_{2α}.

Haemostasis therefore does not seem to be altered significantly by prostaglandins E₁, E₂ and F_{2α}, and thus haemorrhagic or thrombotic complications would not be expected to represent a hazard in their therapeutic use.

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biochemical abnormality in the keratin of mice bearing the dominantly inherited Naked trait.

The mouse phenotype known as Naked (N) has been described elsewhere^{2–4}. In the heterozygote, the hair breaks off close to the root causing patchy depilation which begins around the eyes and spreads to the tail. Before depilation is complete, hair growth begins again on the head, and the mouse grows successive waves of hair passing from head to tail, reflecting the normal moult pattern. Mice homozygous for this mutation, have almost no hair or nails, and usually die within 10 d of birth.

We have investigated the amino acid composition of Nn hair and found it to be significantly different from that of wild type hair. We used mice of the NS-FR strain, inbred by brother-sister mating for 57 generations with forced heterozygosity for the N and albino genes. Eight mice were examined. Four were heterozygous Naked (Nn) and four were homozygous for the normal allele (nn). Each of the groups comprised two black and two albino mice.

Three replicate samples of hair shaft (2 mg each) were taken from each mouse, washed with water and defatted in petroleum ether, dried and added to 5 ml of redistilled 6 N HCl in a hydrolysis tube, which was then evacuated, sealed and heated at 110° C for 24 h. The contents of the tubes were then evaporated under vacuum to dryness and the residues were dissolved in pH 2.2 citrate buffer at a concentration of 0.15 mg of protein ml⁻¹. The amino acid composition of the resulting solution was determined on a Jeol analyser. A standard mixture of amino acid was analysed at every sixth run.

The amino acid composition of the hair samples (Table 1) is expressed as the percentage of moles represented by each amino acid. An analysis of variance was carried out to determine the effect of the N mutation, the albino mutation and the interaction between them. The albino gene causes no significant change nor does it interact significantly with the N mutation. The latter, however, causes two significant changes in amino acid composition a 30.4% reduction in the tyrosine content (3.23 against 4.67 mol %) and a 20.4% reduction in the glycine content (8.41 against 10.51 mol %). Since the probability of finding *n* or more differences, at significance *P* or less among *N* variables is $p^n N! / (N-n)! n!$, the probability that this is a chance finding is 0.00015. A few samples of hair were hydrolysed for 48 and 72 h to estimate rates of amino acid destruction. The corrected values for glycine (mol %) are: normal 9.37, naked 7.72, and for tyrosine: normal 4.58, naked 3.25. The differences between genotypes thus persist when values are corrected for destruction. For these reasons, observed reductions in tyrosine and glycine are almost certainly the result of the mutation.

In 1964, Dedeurwaerder *et al.*⁵ extracted from wool a protein fraction containing 27 mol % of glycine and 11 mol % of tyrosine. The proteins comprising this fraction are now known as the high-gly, high-tyr (HG) proteins. It was apparent from electron microscopic studies of the wool after extraction of the HG proteins, that they are located in the intercellular membrane complex. In fact, the removal of HG proteins releases intact cells from the fibre. A complete sequence of an HG protein has now been published⁶. It has a molecular weight of 6,950 and contains 23% glycine and 19% tyrosine.

It has been shown that the HG protein content of a hair is roughly proportional to its tyrosine content⁷ and, extrapolating from keratin samples whose tyrosine content and HG protein content have both been determined, we estimate that murine hair contains 20–25% by weight of HG proteins. Murine hair contains more tyrosine than any animal hair so far investigated⁸.

The composition of murine HG protein has been determined⁸. It contains roughly three times as much glycine and four times as much tyrosine as whole hair. A defi-

Biochemical marker in dominantly inherited ectodermal malformation

ALTHOUGH several hundred dominantly inherited human malformations have been described¹, the mode of action of the mutant genes responsible for them is, in nearly all cases completely unknown, because of the lack of suitable biochemical markers. Here we describe a well defined

Table 1 Amino acid composition of mouse hair

Amino acid (mol %)	Control				'Naked'				Interaction	F values	
	Black	Albino	Animal	Black	Albino	Interaction	Albino	Naked		Albino	Naked
	1	2	3	4	5	6	7	8			
Lysine	3.00	2.97	3.43	3.30	3.60	4.13	3.63	3.96	2.47	1.81	9.17§
Histidine	1.03	1.03	1.00	0.97	1.00	1.10	1.03	1.03	0.40	1.60	1.60
Ammonia	11.20	10.30	9.87	10.67	11.90	13.03	9.50	11.00	1.75	4.25	1.69
Arginine	5.10	5.07	5.03	5.47	4.80	4.77	5.00	5.03	0.09	3.35	5.95
Aspartic acid	4.90	4.87	5.13	4.83	4.87	4.97	5.00	4.87	0.23	0.46	0.01
Threonine	4.07	4.10	4.10	3.97	4.20	4.13	4.40	4.13	0.95	0.11	4.25
Serine	7.47	7.63	7.10	7.20	7.87	7.17	8.10	7.40	1.58	0.11	1.26
Glutamic acid	11.33	11.53	11.90	11.13	12.20	12.30	12.03	11.70	1.16	0.48	7.27
Proline	5.83	5.86	6.00	5.73	6.10	6.13	6.67	6.50	8.01§	9.24§	38.25†
Glycine	10.33	10.47	10.53	10.73	8.13	8.50	8.50	8.50	0.05	3.61	370.0*
Alanine	4.00	4.07	4.13	4.07	3.90	3.93	3.93	3.97	0.40	3.60	25.60‡
Half cystine	11.53	11.90	11.13	11.46	12.70	11.03	12.93	12.76	2.58	0.41	3.81
Valine	4.03	4.00	4.20	3.97	4.07	4.27	4.20	4.30	0.01	0.85	3.80
Methionine	0.90	1.10	0.97	0.97	1.33	1.07	0.93	1.00	1.38	2.46	1.38
Isoleucine	2.40	2.30	2.43	2.27	2.50	2.50	2.50	2.47	0.03	0.03	8.26§
Leucine	5.87	5.70	5.97	5.70	5.53	5.70	5.77	5.60	0.01	0.35	2.60
Tyrosine	4.37	4.63	4.67	5.00	3.00	3.10	3.43	3.40	0.02	10.14§	170.0*
Phenylalanine	2.53	2.37	2.47	2.47	2.20	2.30	2.37	2.33	0.71	1.40	103.1§

Studies were performed on two mice in each genotype category. The values represent the means of three replicate analyses. F values are with 1 and 4 degrees of freedom in numerator and denominator respectively.

* $P < 0.001$.

† $0.001 < P < 0.005$.

‡ $0.005 < P < 0.01$.

§ $0.025 < P < 0.05$.

ciency of HG protein would therefore explain the low tyrosine and glycine contents of naked hair. A consideration of other amino acids is also consistent with an HG deficiency. There is twice as much phenylalanine in HG protein as in whole hair and there is a deficiency of this amino acid significant at the $P=0.05$ level, in naked hair. On the other hand there is less leucine, lysine and proline in HG than in whole hair and the relative amounts of these amino acids are increased in naked hair ($P<0.05$, <0.05 and <0.005 , respectively). The amino acids which are present in HG protein and in whole hair, in roughly equal amounts are unchanged in naked hair, for example, histidine. We can make a rough estimate of the amount of HG protein which would have to be removed from normal hair. If normal hair, mutant hair and HG protein contain respectively n , m and h mol % of a given amino acid, then:

$$(n-m)/h-m=p,$$

where p is the proportion of HG protein lost⁹. The mean value of p obtained by considering several amino acids is 0.12. Thus, an amount of HG protein equal to about 12% of the total hair by weight is missing in the naked mutant.

Since the HG fraction is very heterogeneous⁸, this almost certainly represents the loss of several molecular species.

The original reason for investigating this mutation in the mouse was its close resemblance to the dominantly inherited human disease, hydrotic ectodermal dysplasia¹⁰. The amino acid changes in this disease are quite different, however. Ectodermal dysplasia hair contains less cystine, proline and serine and more tyrosine and phenylalanine than normal hair and its glycine content is unchanged. It is probably caused by the loss of a matrix component¹¹⁻¹³. The entirely different nature of the two superficially similar phenotypes underlines the danger of accepting animal diseases as models for human ones without a knowledge of the underlying mechanisms.

The results also demonstrate the advantage of using isogenic, animal strains. The amino acid composition of human hair, both normal and dysplastic is very variable and the difference between them is much less clear, presumably as the result of the action of modifying genes. The genotypes of the mice, on the other hand, differ only at the locus whose effects we wish to observe.

Meanwhile, an exploration of the protein fractions in the Naked mouse hair and comparison with those in normal

hair should further elucidate the gene action in this mutation.

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DNA polymerase activity determined by the ultraviolet-protecting plasmid, R-Utrecht

CERTAIN plasmids, including the colicin factor *ColIb* and the drug resistance transfer factor R-Utrecht, reduce the susceptibility of wild-type cells of *Salmonella typhimurium*

to the lethal effects of ultraviolet irradiation while at the same time increasing their susceptibility to the mutagenic effects^{1,2}. These alterations in ultraviolet response may result from plasmid-borne genes whose products participate in repair of single-strand gaps in DNA (ref. 2). Here I present evidence which indicates that at least part of the ultraviolet protection conferred on wild-type cells by R-Utrecht can be attributed to a plasmid-coded DNA polymerase which participates in repair of single strand gaps.

The strains used are derivatives of *S. typhimurium* strain M7471, which is of the 'FIRN' wild-type and differs from LT2 by absence of fimbriae and failure to ferment inositol and rhamnose³. The parent strain, SL4525, has the following genotype: M7471 (*ColEI*-30) *leu*-1051 *malB*479 *cysIII*73 *hisC*527 (amber) *gal*-459. Strain SL4702 is a derivative of SL4525 detected as a clone which failed to release colicin E1. This strain shows increased sensitivity to ultraviolet and ionising radiation because of a deficiency in the enzyme DNA polymerase I, and the mutation it carries has been designated *polA*1 (ref. 4). Strains SL4525/R-Utrecht

and SL4702/R-Utrecht were obtained by selection of clones resistant to tetracycline following conjugation with *S. typhimurium* strain SL1156/R-Utrecht (an LT2 derivative). The characteristics of this strain and of the R-Utrecht plasmid have been described previously².

Among other properties, the *polA*1 strain of *S. typhimurium* (SL4702) shows reduced ability to effect host-cell reactivation (HCR) of bacteriophages which have been damaged by ultraviolet or ionising radiation (D.G.M., unpublished). Figure 1 confirms this finding, showing that preparations of the *Salmonella* phage ES18 which have been exposed to ultraviolet or γ -irradiation plate with lower efficiency on strain SL4702 than on the wild-type parent strain SL4525. Tests with derivatives of these strains carrying the R-Utrecht factor showed that this plasmid has no detectable effect on the HCR capacity of strain SL4525 for either phage preparation (results not shown), and that this plasmid increases the HCR capacity of strain SL4702 for both ultraviolet and γ -irradiated phages to a level approximately equal to that of the wild-type parent strain SL4525 (Figs 1a and b).

Those results suggest that R-Utrecht provides cells of the *polA*1 strain with a plasmid gene product which can participate in repair of single-strand gaps produced in phage DNA either indirectly as a result of excision of ultraviolet-induced photoproducts or directly as a result of exposure to ionising radiation, and that this gene product is therefore functionally equivalent to the chromosomal *polA*⁺ gene product (DNA polymerase I). On this hypothesis, cells of a *polA*1/R-Utrecht strain of *S. typhimurium* would be expected to contain an enzyme capable of carrying out the process known as repair resynthesis or repair replication (that is, resynthesising DNA in the region of a DNA strand gap using the intact opposite strand as a template). The chromosomally-determined enzyme believed to be mainly responsible for this process in normal cells is of course DNA polymerase I, an enzyme whose activity can be detected even in crude assay systems and certainly much more readily than either of the other two known cellular DNA polymerases (II and III)⁴⁻⁷.

Assays for DNA polymerising activity were therefore performed on crude extracts obtained by treatment of strains SL4702 (the *polA*1 mutant), SL4702/R-Utrecht, and SL4525 (the *polA*⁺ parent strain). As expected from previous work⁴ the results (Fig. 2) show first that crude extracts of strain SL4702 contain no detectable DNA polymerising activity. They also show that the derivative of strain SL4702 carrying R-Utrecht has about 25% of the DNA polymerising activity found in extracts of the wild-type parent strain SL4525. It therefore seems clear that a new DNA polymerising activity is associated with the acquisition of R-Utrecht by strain SL4702. Further experiments have shown that the continued presence of R-Utrecht is necessary for this activity to be found in cells derived from strain SL4702, since spontaneous R⁻ segregants of the R⁺ strain have no detectable polymerase activity.

It has been assumed so far that the enzyme which contributes to DNA repair in *polA*1 cells carrying R-Utrecht and which is responsible for the DNA polymerising activity found in such cells is directly coded by the plasmid. There are other possibilities, however. For example, the effect of the plasmid could be to derepress a chromosomal gene specifying a DNA repair enzyme which has DNA polymerase activity. If so, the chromosomal gene most likely to be implicated is the *polA* gene itself, since detection of either of the other two known cellular DNA polymerases (II and III) in the crude assay system used here is improbable. It then becomes necessary to assume that the *polA*1 mutation in strain SL4702 leaves the *polA* gene product with some residual DNA repair and polymerising activity which can be amplified by plasmid-directed derepression. In fact, it is necessary to extend this assumption

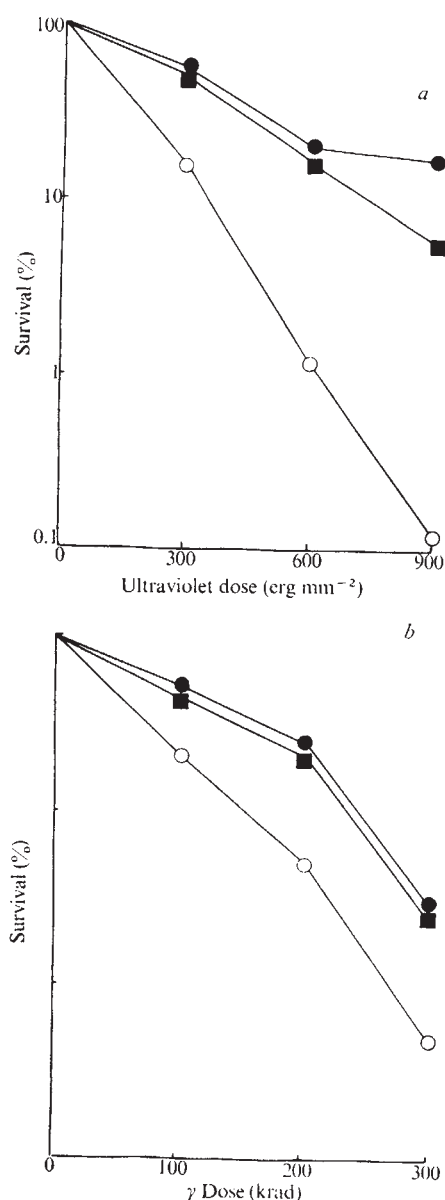


Fig. 1 Host-cell reactivation of preparations of bacteriophage ES18 exposed to, *a*, ultraviolet irradiation and, *b*, γ -irradiation. Phage suspended in saline were irradiated, diluted in broth and titrated on the various strains by the drop-on-lawn method⁸. ■, SL4525 (POL⁺); ○, SL4702 (*polA*1); ●, SL4702/R-Utrecht (*polA*1/R-Utrecht).

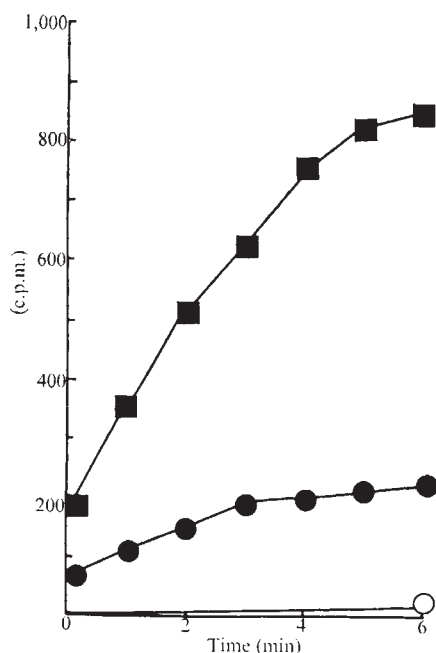


Fig. 2 Assays for DNA polymerase activity of strain, ■, SL4525 (POL⁺), ○, SL4702 (*polA1*), and, ●, SL4702/R-Utrecht. Bacteria grown on the surface of a solid medium were suspended in 0.1 M Tris, 0.01 M MgSO₄ (pH 7.4) at a concentration of about 1×10^{10} ml⁻¹. Crude extracts of these suspensions were then prepared by sonication in a 150 W MSE ultrasonic disintegrator. The resulting extracts were centrifuged at 1,000g for 10 min. To 0.9 ml of each supernatant, 0.1 ml sonicated calf thymus DNA was added (final concentration 20 μ g ml⁻¹), and, 5 min later 0.3 ml triphosphate solution (final concentrations 100 nmol ml⁻¹ dGTP, dCTP, dATP and 0.6 nmol ml⁻¹ ³H-TTP). Samples of 0.02 ml were taken from each reaction mixture and dried on Whatman No. 1 filter paper disks (2.5 cm diameter). The disks were immediately placed in cold 5% trichloroacetic acid, 1% sodium pyrophosphate for at least 2 h then rinsed with 95% ethanol, dried, and counted in a scintillation counter.

to four other independently-isolated *polA* mutants of *S. typhimurium* since R-Utrecht has similar effects in all five strains. It seems equally improbable that a plasmid-determined suppressor of any known type could explain the effects of R-Utrecht in the five different *polA* mutants, especially since one of the mutants (*polA3*) is known to be suppressible by ochre and amber suppressors whereas the others are known not to be (D. G. M., and M. R. Beazer, unpublished).

On balance, therefore, it seems highly likely that the R-Utrecht plasmid codes directly for a DNA polymerase which resembles the chromosomal DNA polymerase I in being readily detectable in assays performed on crude cell extracts and in contributing to repair of single-strand gaps in DNA. Such an enzyme presumably provides R⁺ derivatives of wild-type strains of *S. typhimurium* with additional DNA polymerising activity, and thus may account, at least in part, for their increased resistance to the lethal effects of ultraviolet and ionising radiation. This would seem to be a reasonable assumption, since there is evidence from other studies that strains of *S. typhimurium* which have increased levels of DNA polymerase I activity (probably as a result of mutation of chromosomal genes in this case) show increased resistance to both ultraviolet and ionising radiation⁸.

Plasmid-coded DNA polymerases may well have other functions quite apart from their role in DNA repair. For example, they may be responsible for the special type of DNA replication (known as transfer replication) which is thought to be involved in the transfer of plasmid (and in some cases chromosomal) DNA from a donor to a recipient

cell during conjugation⁹, or alternatively they may be involved in some aspect of the replication and stable maintenance of plasmid DNA within the bacterial cell. In the latter situation, possible roles which might be attributed to plasmid-coded DNA polymerases will depend upon whether they resemble the multifunctional DNA polymerase I of *Escherichia coli* in properties other than just ease of detection in crude assay systems (for example, in having exonuclease activities as well as their polymerase activity). Thus, until a particular plasmid enzyme has been studied in detail, the exact role (or roles) which it plays in the cell must remain a matter for speculation.

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Altered rate of DNA replication in ageing human fibroblast cultures

SINCE the demonstration that diploid human fibroblasts have a limited lifespan in culture^{1,2} and that the lifespan in culture is a function of the age of the donor^{3,4}, many explanations of fibroblast ageing have been offered⁴. The production of error-containing enzymes by either an 'error catastrophe'^{5,6} or somatic mutation has been suggested as one possible cause^{4,7}. Errors in the enzymes which replicate the cellular DNA might have the gross effect of slowing the rate of DNA replication. Here we examine the rate of DNA chain elongation and the distance between DNA synthesis initiation sites in senescent and control fibroblasts by DNA fibre autoradiography.

Asynchronous cultures established from the male foetal lung fibroblast strain MRC-5⁸ were pulse-labelled with ³H-thymidine and prepared for DNA fibre autoradiography⁹⁻¹¹. Parallel experiments were performed with a visibly senescent culture (culture passage 58) and a control nonsenescent culture (passage 16). The strain MRC-5 generally becomes visibly senescent after about 50 passages and ceases growth after 60-70 passages⁷.

Tandem arrays of grain tracks, similar to those observed in other eukaryotic systems⁹⁻¹³, were observed in autoradiographs prepared from both passage 58 and passage 16 cells. The length of individual tracks in these arrays from cells labelled for 10, 20 and 30 min is shown in Fig. 1. For each of the three pulse experiments, the length of tracks from the passage 58 cells were significantly smaller ($P < 0.05$) than those from passage 16 cells. The average track lengths for passage 58 and passage 16 cells were respectively: for the 10 min pulse, 7 μ m and 10 μ m;

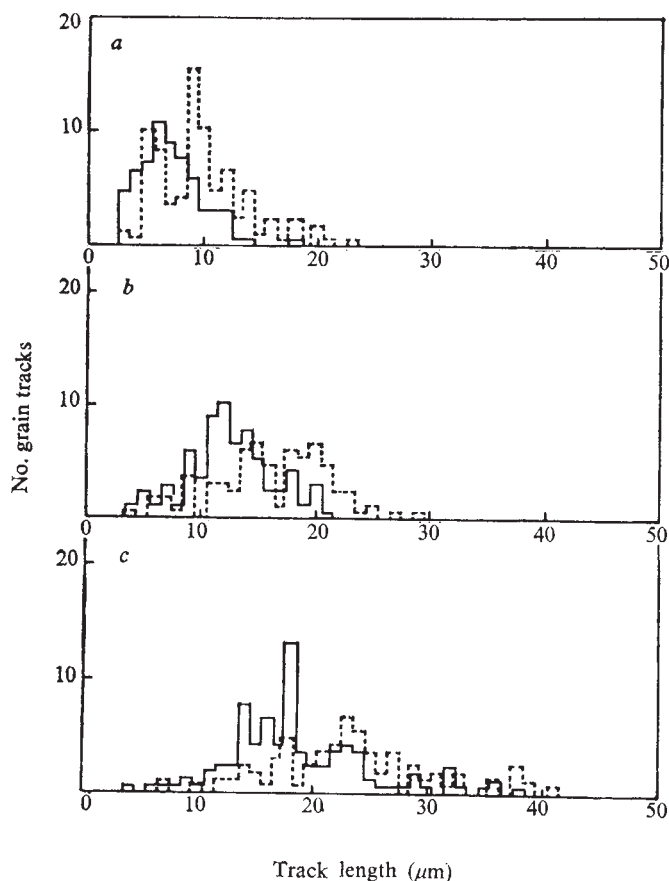


Fig. 1 Histograms of lengths of labelled segments of DNA from senescent and non-senescent cultures. Methods for labelling the DNA and fibre autoradiography followed procedures of Lark *et al.*¹¹ and Huberman and Tsai¹⁶ with minor modifications. Cultures of MRC-5 (passage 58 and passage 16 cells previously tested for mycoplasma contamination) were seeded before labelling into 50 mm plastic Petri dishes containing 10 ml of Eagle's basal medium (BME), supplemented with 10% foetal calf serum, nonessential amino acids, and penicillin (100 units ml⁻¹), streptomycin (100 μg ml⁻¹). Approximately 10⁶ cells were labelled in 2 ml of the above medium containing 200 μCi of ³H-thymidine (56 Ci mmol⁻¹, Amersham) for various pulse times (10, 20, 30 min, 1 h or 3 h). All pulses were done at 37° C. The pulses were terminated by washing with cold isotonic saline containing fluorodeoxyuridine (0.1 μg ml⁻¹) and the cells removed by trypsin (0.125% with 0.1 μg ml⁻¹ fluorodeoxyuridine) treatment at 37° C for 15 min (ref. 16). The DNA from approximately 5,000 cells was spread on glass slides^{11,16}. The slides were rinsed with 5% trichloroacetic acid and ethanol^{11,16} and covered with Kodak AR-10 stripping film¹⁰. Exposure time was 3–4 months. Track lengths were measured with an ocular micrometer. Slides were coded before measurements to exclude the possibility of bias in scoring. —, Passage 58; ----, passage 16. *a*, 10 min pulse; *b*, 20 min pulse; *c*, 30 min pulse.

for the 20 min pulse, 12 μm and 16 μm; and for the 30 min pulse, 19 μm and 23 μm. The tracks labelled in 1 h and 3 h labelling periods were very long, often exceeding 100 μm in length, in autoradiographs from both passage 58 and passage 16 cultures. These were not further characterised.

The length of grain tracks produced during a pulse time which is short relative to the time necessary to complete replication of a replicating unit is primarily a function of the rate of chain elongation of DNA (ref. 10). The results, therefore, suggest that DNA chain elongation is slower in senescent cells. It is unlikely that the difference in track lengths represents differential stretching of DNA during preparation of autoradiography since the grain density of autoradiographs from passage 58 and passage 16 cells was not significantly different. It is also unlikely that the

difference in track lengths represents a difference in the time required for equilibration of precursor pools. No measurable gradients of grain density were observed in grain tracks from either passage 58 or passage 16 cells. The difference in track lengths probably does not reflect an age-independent difference in the rate of DNA synthesis of different sublines of cells. Independent experiments with different sublines of senescent (passage 60) and non-senescent (passage 20) cells showed the same difference in the rate of DNA chain elongation (T.D.P., unpublished). It should be pointed out that a 25% reduction in the rate of DNA chain elongation in senescent cells does not necessarily imply that the period of DNA synthesis (the S period) is 25% longer in senescent cells. Preliminary measurements of the length of the S period (R.A.F., G.M.T., T.D.P., unpublished) have indicated no difference between senescent and non-senescent cells.

The rate of chain elongation as estimated by the length of grain tracks produced in a pulse-labelling experiment is only an approximation of the true rate of synthesis^{10,12}. Initiation of DNA synthesis, termination of DNA synthesis and fusion of neighbouring replication units during the pulse are possible sources of error. Nevertheless, the calculation of the replication rate from the 10 min pulse experiment suggests that the rate of DNA chain elongation in non-senescent human diploid fibroblasts, 0.5 μm min⁻¹, is similar to the rate of DNA synthesis in other mammalian systems^{9,10,13}. For this calculation, it was assumed that DNA synthesis in human diploid fibroblasts is bidirectional from each initiation site as in other eukaryotic systems^{10,12}.

The distance between initiation sites along the chromosome was measured by determining the centre-to-centre distance between adjacent tracks within a tandem array of tracks¹⁰. There was no significant difference in the spacing of these sites in passage 58 and passage 16 cells (Fig. 2). Most of the centre-to-centre distances were between 15 μm and 60 μm. Similar distances have been found in Chinese hamster ovary cells¹⁰.

One possible reason for the slower rate of DNA elongation is that a proportion of the enzyme molecules in enzymes required for DNA replication is defective in senescent cells. Holliday and Tarrant⁷ reported that senescent cultures of MRC-5 contained as much as 25% heat labile glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and suggested that the heat lability was the result of misincorporation of amino acids during protein synthesis. If such misincorporation occurs in DNA polymerase, then even a small proportion of altered molecules could have a significant effect on the rate of chain elongation. One molecule of DNA polymerase at the replication fork may synthesise a small stretch of DNA (ref. 14) (for example, one Okazaki fragment). An occasional defective

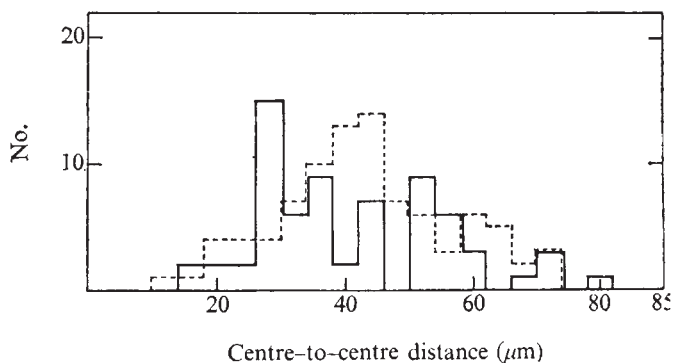


Fig. 2 Histogram of the centre-to-centre distances between adjacent tracks in a tandem array. The autoradiographs were from the experiment described in the legend to Fig. 1. The centre-to-centre distances did not differ in the 20 min and 30 min pulses and the data from these experiments were pooled. —, Passage 58; ----, passage 16.

polymerase molecule which binds normally may block replication for a significant length of time before being replaced by a normal molecule. Faulty DNA polymerase molecules would also be expected to have 'mutator' activity⁷; errors in replication would accumulate irreversibly and may be a major cause of cell death.

The slower rate of chain elongation in senescent cells is not necessarily the result of defective DNA polymerase molecules. It might also reflect smaller pools of DNA precursors or energy limitations. Slow DNA replication as a consequence of limited precursor pools could be detrimental to the cell, since it has been observed that thymine-requiring strains of *B. subtilis* grown in limiting concentrations of thymine have a mutation rate several orders of magnitude higher than the background rate¹⁵. Slow DNA synthesis may also be a more general factor in cell death. For example, completion of synthesis of certain replicons may be required to trigger other essential events in the cell cycle. Slowing the rate of DNA synthesis could disrupt these control mechanisms.

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Control of translation of globin mRNA in embryonic cells

WHEN mouse or rabbit globin mRNA is injected into oocytes of *Xenopus laevis* both α and β mRNAs are translated but only about one fifth as much α globin is synthesised as β globin¹⁻³. On the other hand, the same message sample results in about equal amounts of α and β globin synthesis in a reticulocyte cell-free system and is believed to contain roughly equal amounts of α and β globin mRNA. Giglioni *et al.*³ have shown that if haemin is injected into oocytes at the same time as globin mRNA, there is an equal ratio of α : β globin synthesis, and their experiments strongly suggest that this is achieved by increasing the efficiency of translation of α globin mRNA. These experiments thus identified a factor which has a different effect on two kinds of mRNA, and further demonstrated that the microinjection of living cells can

reveal regulatory effects not readily seen in the cell-free systems used so far. The experiments reported here were also carried out by injecting mRNA into frog cells. They show that during development a component is formed that alters the ratio of α : β rabbit globin synthesis, a change not seen when mouse globin mRNA is injected. This is therefore a second example of a translational factor revealed by a living cell assay system. This component shows an even higher degree of selectivity in its action than haemin.

Rabbit or mouse globin mRNA is translated into globin when injected into fertilised frog eggs⁴⁻⁶, which differ in several respects from oocytes. Mouse mRNA yields a low, although somewhat variable, ratio of α to β globin synthesis in eggs and embryos of all stages (Table 1), as it does in oocytes (Fig. 1). There is no proof that the extremely low mouse α : β ratios seen in eggs and morulae are significantly different from the low ratios seen in other stages. Rabbit globin mRNA also yields a low ratio of α : β globin when translated in unfertilised or newly fertilised eggs; on the other hand, this ratio changes progressively, in the absence of injected haemin, to an almost equal synthesis of α and β globin as the embryos develop (Table 1). The ratio of α : β globin synthesis approaches one, when tested in embryos at

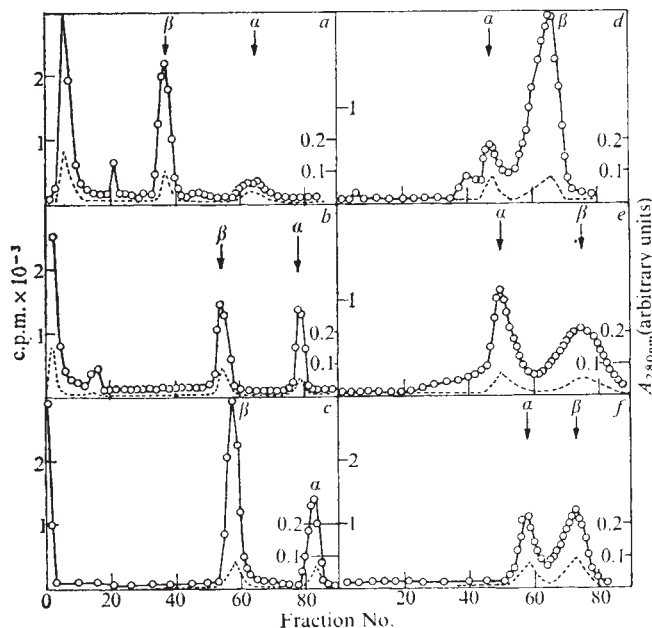


Fig. 1 Effect of haemin on globin synthesis in oocytes injected with saturating and subsaturating amounts of rabbit and mouse globin messengers. A stock of 16 mM haemin was prepared by the method of Zucker and Schulman¹⁰ except that the final solution contained 90% (v/v) ethylene glycol. Oocytes were injected with mRNA dissolved in saline¹, the latter containing haemin (1.6 mM) where indicated: the intracellular concentration of haemin would have been about 80 μ M. Globin was prepared from whole oocytes and was fractionated by carboxymethyl cellulose chromatography². $\circ$ — $\circ$, c.p.m. per fraction; — — —, absorbance at 280 nm of added marker α and β globin chains. *a*, globin synthesis (α : β =17%) in oocytes injected with mouse mRNA (12 ng per cell); *b*, the effect of haemin (α : β =84%) on synthesis directed by such subsaturating amounts of mouse mRNA; *c*, the effect (α : β =46%) of haemin on saturating amounts (95 ng per cell) of mouse mRNA; *d* (α : β =12%) and *e* (α : β =84%) (+haemin) show analogous experiments with subsaturating rabbit mRNA (24 ng per cell), while *f* shows globin synthesis (α : β =55%) with saturating rabbit mRNA (48 ng per cell) plus haemin. The α : β globin synthesis ratio (α : β =20%) in oocytes injected with saturating messenger and no haemin is similar to that seen in oocytes injected with subsaturating mRNA. Oocytes injected with buffer containing no RNA synthesise, no globin like species.

Table 1 Ratio of α : β -globin synthesis in mRNA-injected oocytes and eggs

Hours after fertilisation	Oocyte (ovarian)	Oocyte (matured)	Unfertilised egg	Morula	Blastula	Gastrula	Neurula	Post-neurula	Early tail bud	Tail bud	Heart beat	Swimming tadpole	Stage 45
			0	4	8	14	20	26	44	50	54	72	180
Mouse globin mRNA	20	—	3	5	24	10	10	20	14	15	11	11	—
Rabbit globin mRNA	17	20	19	22	67	59	60	68	66	—	—	54	54

Figures show ^3H - α -globin radioactivity as a percentage of ^3H - β -globin synthesis. Oocytes and eggs were injected with mouse (12 ng per cell) or rabbit (7–20 ng per cell) globin mRNA^{1,5}. After the appropriate time, cells were labelled with ^3H -histidine by incubation (oocytes) or injection (embryos). Embryo homogenates were treated with acid acetone, and the resulting globin was chromatographed on a carboxymethylcellulose column². Matured oocytes were obtained by progesterone treatment (10 $\mu\text{g ml}^{-1}$) of immature oocytes.

and beyond the neurula stage (20 h after fertilisation, Table 1).

Haemin can restore the efficient translation of rabbit and mouse α -globin mRNA in oocytes; but unlike the *Xenopus* embryo factor, it is equally effective with mouse and rabbit globin mRNA (Fig. 1). For this reason the *Xenopus* factor is probably not itself haemin, though it may operate by part of the same mechanism since haemin and the embryo factor seem not to have an additive effect on rabbit globin synthesis in neurulae.

Some information is available as to why less α globin is made than β globin. For example this effect does not result from differential breakdown of completed α chains because α and β peptides are present in the usual 1:5 ratio in the polysomes of mRNA-injected oocytes⁷. It also cannot be explained in a simple way by destruction of α rather than β mRNA because haemin increases the level of α -globin synthesis even when injected as long as 48 h after the mRNA (Table 2). It seems likely therefore that the effect is a result of a true translation control phenomenon.

Two explanations of the haemin effect seem plausible. One is that haemin raises the efficiency of initiation of α but not β -globin mRNA. Alternatively haemin might remove some restriction to ribosome transit⁷ located about half way along the α -globin mRNP particle. Neither model necessitates a requirement for specific factors in the translation of specific messages and both models can be used to describe the agent enhancing rabbit α -globin synthesis during development. Since haemin and the developmental factor differ in their effects on mouse globin synthesis, however, their mechanism of action cannot be identical. The significance of these two regulatory phenomena is not clear. In man, genetic deficiencies in haem synthesis are commonly associated with low globin synthesis and a low (0.71) ratio of α : β -globin synthesis^{8,9}. Haem may therefore be involved in α -globin mRNA translation in normal reticulocytes. The principal importance of the embryo factor, whose developmental significance is unknown, is that it indicates the possible existence in cells of a wide

range of natural substances which may have very selective effects on message translation; the purification of those may help to reveal the kinds of mechanism by which translation can be controlled.

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Table 2 Effect of haemin when supplied after the initial injection of globin mRNA

Haemin injection (h after message)	Period of labelling (h after message)	α : β (as a %)
No haemin	1–16	17
0.15	1–16	93
Simultaneous	1–16	84
No haemin	48–64	25
Simultaneous	48–64	83
48	48–64	57

Oocytes were injected with mouse globin mRNA (12 ng per cell) and were then injected with saline¹ or haemin. The cells were labelled with ^3H -histidine. Globin was prepared and fractionated². A further experiment was carried out by labelling oocytes for 5–18 h, with similar results to the 48–64 h experiments shown, except in the case of 'no haemin' samples. About 30 ml of a 1.6 mM solution of haemin was injected per oocyte so as to give an intracellular concentration of about 80 mM.

Distinct haematological disorder with deletion of long arm of No. 5 chromosome

So far, the only specific chromosome abnormality in haematological disorders is the Ph1-chromosome. Chromosome abnormalities have been described in so-called idiopathic sideroblastic or refractory anaemias¹ but a constant cytogenetic abnormality associated with a defined clinical syndrome has not been reported.

Out of a series of patients with refractory anaemia or anaemia of-ill determined origin, three patients, a 46-yr-old male, and two females respectively 40 and 75 yr of age, were found to present a clinically very similar haematological disorder and an apparently identical chromosomal anomaly in the bone marrow.

The haematological disorder is characterised by longstanding aregenerative slightly macrocytic anaemia with haemoglobin values ranging between 6.5 and 10 g dl, low to normal leukocyte count (3,000–4,000) and normal to elevated platelets (300,000–500,000). The bone marrow showed erythrocytic hypoplasia with evidence of disordered maturation and dyserythropoiesis, normal myeloid series but nevertheless moderate excess of blast

cells (up to 15%), and absence of lobulation of the megakaryocytic nucleus. Patients with a similar haematological syndrome have been described in the past² but no cytogenetic investigation was performed.

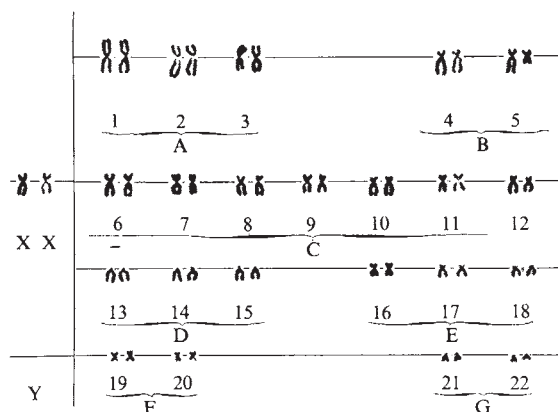


Fig. 1 Routine karyotype (orceine) from a 48-h bone marrow culture of one of the patients.

The chromosomal anomaly is a deletion of approximately two-thirds of the long arm of a chromosome No. 5, without translocation of the deleted segment (Figs 1 and 2), present in 75–90% of the bone marrow cells cultured without PHA for 24–48 h. No single abnormal cell has so far been found in peripheral blood cultured with PHA and in skin fibroblasts (Table 1); no metaphases were obtained from lymphocyte cultures without PHA. The deletion seems to be interstitial and the following bands may be involved: 5q 12–5, 5q 21–3. The association of this chromosomal anomaly with a defined haematological disorder may represent a newly recognised syndrome. To our knowledge one case has been reported with similar clinical course and chromosome anomaly, but the B chromosome involved was not identified³.

The absence of the deleted chromosome in the lymphocytes of the peripheral blood cultured with PHA, and in skin fibroblasts of our phenotypically normal patients indicates that the anomaly is an acquired one, occurring originally in a bone marrow stem cell, followed by clonal evolution and gradual replacement of an important part of the bone marrow.

As far as can be concluded from the cytogenetic investigations, the abnormal cells are confined to the bone marrow. It is possible that the stem cell with the abnormal chromosome is capable of erythroid, myeloid and megakaryocytic differentiation, but at present only morphological arguments can be found in favour of this hypothesis: an erythroid hypoplasia, megakaryocytic nuclear anomaly, and a slightly reduced number of granulocytes

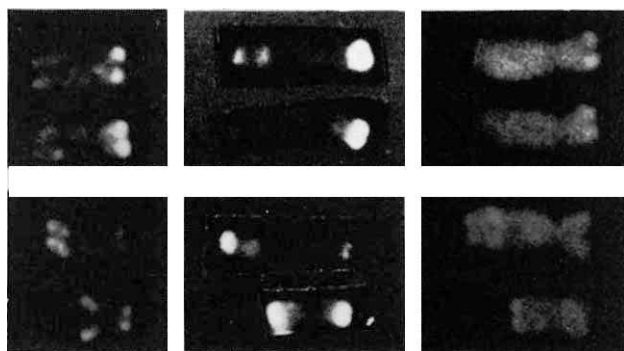


Fig. 2 The B chromosomes of the three patients (T banding) showing that the chromosome involved is a No. 5, of which most of the long arm is missing.

Table 1 Summary of the cytogenetic investigations

Patients and tissues examined	No. of cells with deletion 5q (46 cells only)		
	Present	Absent	Undertermined
M.G. (1) Marrow 48 h 1971	5	—	13
(♀, 40 yr) (2) Marrow 48 h January, 1974	17	1	3
(3) Marrow 48 h February, 1974	94	7	7
(4) Skin Fibroblasts	—	30	—
E. M. (1) Marrow 48 h September, 1973	98	18	8
(♀, 75 yr) (2) Marrow 48 h March, 1974	87	15	15
(3) Blood + PHA, 72 h	—	50	—
(4) Blood + PHA, 72 h	—	50	—
B.A. (1) Marrow 48 h February, 1974	67	29	7
(♂, 46 yr) (2) Blood + PHA, 72 h February, 1974	—	22	—

* No growth was observed in peripheral blood cultured without PHA.

with moderate blast excess in the marrow. This situation could be very similar to that found in Ph1-positive disease. Here also the chromosomally abnormal stem cell is capable of myelocytic, erythroid or megakaryocytic differentiation, and the extent of abnormal proliferation may be different for each cell line, for example, although the myeloid series is most often affected, polycythemia or thrombocytosis as well as anaemia or thrombopenia can be the predominant haematological features. So far there is no explanation for the variation in the haematological features of the Ph1-positive conditions. Examination of a larger group of patients presenting with the 5q syndrome will be required before the extent of possible variability in the haematological features of this syndrome can be assessed.

Finally it is to be noted that according to Price *et al.*⁴ one of the haemoglobin structural genes may be located on the long arm of a B chromosome. If their findings are confirmed, and if the B chromosome turns out to be a No. 5, a possible relationship between the deleted chromosome and the anaemia must be considered.

More details about these patients will be published in a forthcoming paper. H.V.D.B., was supported by a grant from FWGO and J.-J. C. and G. D., by NFWO.

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Cytophotometric factors causing apparent differences between Feulgen DNA contents of different leukocyte types

A CENTRAL issue in the study of both metazoan differentiation and the control of cellular activity is the validity or otherwise of the hypothesis^{1,2} that nonreplicating nuclei of a given higher organism contain a constant amount of DNA per haploid set of chromosomes. Differential elimination or increase of DNA has been demonstrated in dipteran polytene chromosomes³, and in the oocytes of *Acheta* and amphibia⁴, but apart from these possibly special cases the most studied apparent exception to the constancy rule is probably provided by mammalian leukocytes⁵⁻¹¹. Most workers in this field have found the apparent DNA content of the relatively large nuclei of monocytes, as assessed by Feulgen cytophotometry, to be between 5% and 20% higher than that of the smaller, more darkly stained nuclei of lymphocytes and polymorphonuclear leukocytes. The consensus of opinion seems to be that the measured differences are probably due to nonstoichiometry of staining rather than to genuine differences in the amounts of DNA present. But work published to date has in general been performed without certain technical refinements which have recently become available, and we have now investigated the possibility that systematic cytophotometric errors may have been responsible for at least some of the reported differences between cell types.

Air-dried smears of normal adult human blood were

bandwidth (about 50 nm at total cutoff) was negligible under the stated conditions. At least 10 measurements, each accompanied by a blank scan of empty background, were made of each cell under each set of conditions. At least eight nuclei of each cell type studied (lymphocytes, monocytes and polymorphs) were measured on a single stained slide in each of two experimental series.

Both series gave essentially similar results, those of the series with 4% glare being given in Table 1. It will be seen that with glare and residual distribution error uncorrected, the mean integrated absorbance (that is, apparent Feulgen DNA content) of monocytes was significantly higher than that of lymphocytes, which was in turn significantly higher than that of polymorphs. When glare but not residual distribution error was corrected the difference between monocytes and lymphocytes disappeared, but both had significantly higher apparent Feulgen DNA contents than did polymorphs. With correction for residual distribution error but not glare, lymphocytes and polymorphs had similar apparent Feulgen DNA contents, which were, however, lower than that of monocytes. No significant difference between the three cell types was found if glare and residual distribution error were both corrected.

The results suggest that in our uncorrected measurements glare is largely responsible for lymphocytes having a lower apparent Feulgen DNA content than monocytes, and that residual distribution error together with glare accounts for the still lower apparent Feulgen DNA content of polymorphs. This interpretation is consistent with the mor-

Table 1 Feulgen-DNA content of different types of leukocyte

	Uncorrected	Integrated absorbance at $\lambda_{\max}$ Corrected for residual glare	Corrected for residual distribution error	Corrected for both residual glare and distribution error
Lymphocytes (L) <i>N</i> =9	19.24±0.25	21.67±0.15	19.46±0.28	21.89±0.21
Polymorphs (P) <i>N</i> =9	18.54±0.19	20.98±0.11	18.84±0.21	21.62±0.10
Monocytes (M) <i>N</i> =8	20.18±0.23	22.03±0.17	20.18±0.21	22.06±0.23
	L < M; <i>t</i> =2.76‡ L > P; <i>t</i> =2.23‡ P < M; <i>t</i> =5.50§	L < M; <i>t</i> =1.59* L > P; <i>t</i> =3.71§ P < M; <i>t</i> =5.19§	L < M; <i>t</i> =2.06† L > P; <i>t</i> =1.78* P < M; <i>t</i> =4.51§	L < M; <i>t</i> =0.55* L > P; <i>t</i> =1.16* P < M; <i>t</i> =1.75*

* Not significant.

† *P*<0.06.

‡ *P*<0.05.

§ *P*<0.01.

$\lambda_{\max}$ was about 560 nm. Results are given as mean integrated absorbance (in μm^2 units) ± s.e.

fixed in methanol-formalin-acetic acid (80:10:5) for 1 h at room temperature, washed in running tap water for 60 min, hydrolysed in 5 N HCl at 25°C for 80 min (found in pilot experiments to give maximum staining intensity), rinsed in distilled water, stained 40 min in fresh Schiff's reagent (prepared by the de Tomasi method¹²), rinsed in distilled water, dehydrated in a series of alcohols and mounted in Polymount. Cytophotometry at $\lambda_{\max}$ was performed using a Vickers M86 scanning microdensitometer with a 1.3 NA ×100 apochromatic objective. The achromatic microscope condenser was used dry at full aperture. The field illuminated was limited by a field stop to reduce glare; each nucleus was measured both with and without electronic compensation¹³ for the measured residual glare, which was 4% in a series of experiments with a 40 μm diameter illuminated field, and 6% in a series with a larger illuminated area. Two sizes of flying spot, respectively 0.48 μm and 0.95 μm diameter in the object plane, were used to permit correction for residual distribution error¹⁴. The monochromator entrance slit was opened as far as possible to increase the signal/noise ratio; it could be shown (methods and results to be published) that chromatic error due to the relatively wide monochromator

phological features of the three types of cell nucleus, since stained lymphocyte nuclei have a higher mean absorbance than those of monocytes (and are therefore more liable to be erroneously measured in the presence of glare), while polymorph nuclei have both a high mean absorbance and a less uniform distribution of chromatin than either monocytes or lymphocytes.

We believe that uncorrected glare and distribution errors account for at least some of the previously reported apparent differences in the Feulgen DNA contents of different types of leukocyte, and that there is therefore no longer any compelling reason to postulate either genuine differences in the amounts of DNA present, or nonquantitative staining of DNA by the Feulgen method.

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C-type viral particles in a urinary bladder neoplasm induced by *Schistosoma haematobium*

NEOPLASMS may be induced in nonhuman primates after infection with *Schistosoma haematobium* (refs 1 and 2 and unpublished results of R. E. K., A. W. Cheever, B. J. Myers, S. W. Young and J. A. Moore). After various times, masses develop within the urinary bladders of the hosts, ranging from hyperplasia to papillary carcinoma as determined histopathologically. Although the basic mechanism of tumour induction in such cases is not known, the many factors involved could include stimulation of endogenous virus infections. It is therefore interesting that we have now found C-type viral particles in one out of four papillary carcinomas of capuchin (*Cebus* sp.) monkeys experimentally infected with *S. haematobium*. None were seen in two infected animals that developed only hyperplasia and squamous metaplasia. Previous examination of normal bladder tissue has failed to demonstrate similar C-type viruses. For electron microscopy and infection of the animals with *S. haematobium* we used published methods^{1,2,4}. The bladders were taken from monkeys previously infected with *S. haematobium* (Table 1). Histological evaluation based on examination of biopsy samples is also provided (Table 1).

Table 1 Histological evaluation of bladders of capuchin monkeys infected with *S. haematobium*†

Animal No./sex	No. of cercariae inoculated	Duration of infection (weeks)	Egg passage* Faeces	Urine	
CA 23 M	2,000	113	+	++	Hyperplasia
CA 62 F	2,000	109	0	+	Slight hyperplasia, and squamous metaplasia
CA 64 M	1,000	109	+	0	Papillary carcinoma
CA 67 M	2,000	109	++	+	Papillary carcinoma and squamous metaplasia
CA 68 F	2,000	109	+	+	Papillary carcinoma and squamous metaplasia
CA 70 M	1,000	111	+	+++	Papillary carcinoma and squamous metaplasia

* +, ++, +++, Indicate few, moderate numbers, or many eggs in samples 1-3 weeks before indicated duration of infection.

† Carried out by Dr A. W. Cheever, National Institute of Allergy and Infectious Diseases.

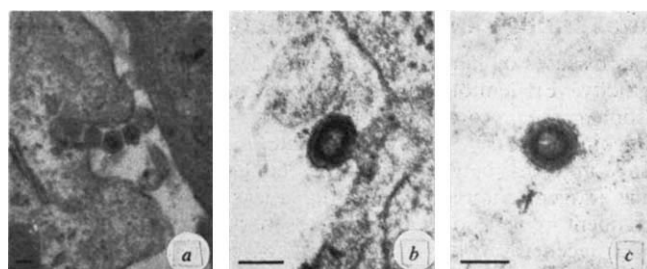


Fig. 1 *a*, Neoplastic transitional epithelium from urinary bladder of monkey No. 70 with typical mature C-type viral particles in extracellular spaces. *b*, C-type particle budding from plasma membrane. *c*, Immature C-type particles in extracellular space. (Scale: 100 nm.)

Figure 1 shows electron microscopic evidence of C-type viruses at various stages of maturation associated with transitional epithelial cells in the bladder carcinoma of capuchin No. 70. This tissue was the same as that examined by light microscopy. Viral particles were readily observable throughout the transitional epithelial areas, but were absent in the loose connective and smooth muscle layers. It should be emphasised that although one of the six monkeys examined was positive, this was the animal with moderate atypia. As Table 1 shows, this animal was also passing the highest number of eggs in its urine, an indication of involvement of the bladder. Control tissue, that is bladders of animals not infected with *Schistosoma* as well as those with minimal or minor involvement of the bladder, has so far failed to disclose the presence of C-type particles.

No definitive conclusions concerning the aetiology of bladder cancer can be drawn from these observations, but they do offer an intriguing approach for further study. Bladder cancers of nonhuman primates are extremely rare³, and while coincidence cannot be ruled out, several other considerations must be examined. It has been suggested that all primates, like some other animal species, contain endogenous C-type particles^{6,7}. Is the schistosome the cause or is it the triggering mechanism? On the other hand, is this tumour development due to an exogenous agent, which is carried by the parasite?

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Ethnicity is a significant factor in the epidemiology of rubella and Hodgkin's disease

SOME diseases of humans have an incidence which varies with ethnicity. Epidemiologic patterns are seen for example in nasopharyngeal carcinoma¹, carcinoma of the liver² and Hodgkin's disease³. There are quite marked differences in HL-A antigens between some ethnic groups; in particular, some antigens commonly found in caucasoid racial groups are infrequent in negroid groups and absent in mongoloid groups⁴.

Our recent study (unpublished) of 61 caucasians (28 males) with congenital rubella revealed an increased incidence of HL-A antigens 1 and 8 (HL-A1: $\chi^2 = 9.57$, P corrected for 25 comparisons (P^c) < 0.05; HL-A8: $\chi^2 = 4.40$, P^c < 0.89), because of the very high occurrence of these antigens in the female subjects (HL-A1: $\chi^2 = 14.32$, P^c < 0.003; HL-A8: $\chi^2 = 9.61$, P^c < 0.048). Although the incidence of antigens 1 and 8 was not raised in the male subjects, those males who possessed these antigens were clinically more severely affected by the disease ($\chi^2 = 4.17$, P < 0.04).

We can now show that ethnic variations in the frequency of HL-A antigens 1 and 8 influence the epidemiology of rubella, and that striking similarities are found in the epidemiology of Hodgkin's disease. These similarities support the suggestion that Hodgkin's disease has an infectious aetiological component but do not necessarily relate it to rubella.

In order to determine whether the frequency of HL-A antigens 1 and 8 is relevant to rubella epidemiology throughout the world, we correlated (Fig. 1) the percentage of young adults with serological evidence of previous rubella infection (that is, % seropositive) with the gene frequencies of HL-A1, HL-A8 and the mean gene frequency of HL-A1 and 8 for that ethnic

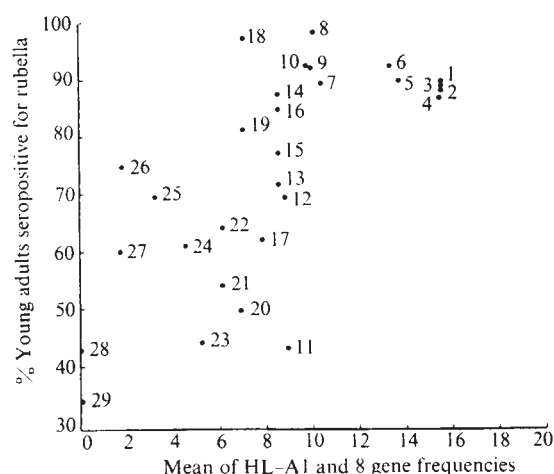


Fig. 1 Geographical correlation between the mean of HL-A1 and 8 gene frequencies and seropositivity to rubella in young adults (aged approximately 20-29). HL-A gene frequencies were taken from ref. 4 except for localities 4, 5, 6, 7 and 26, where refs. 19, 21, 23, 25 and 34 were used respectively. Correlation coefficient (r) = 0.68, t test on r (t_r) = 4.78, P < 0.001. Localities indicated, together with references for the source of the rubella serology data, are as follows: (1)=England¹⁵; (2)=Australia¹⁶; (3)=United States, Houston¹⁷, caucasian; (4)=Sweden, Stockholm¹⁸; (5)=Holland²⁰; (6)=Germany, West Berlin²²; (7)=Soviet Union, Moscow²⁴; (8)=Egypt²⁶, Arabs; (9)=Tunisia²⁷; (10)=Iran²⁸; (11)=Puerto Rico²⁹; (12)=Bermuda³⁰; (13)=Peru, caucasian⁹; (14)=France, Lyons¹⁷; (15)=Brazil, Sao Paulo³¹; (16)=Argentina⁹; (17)=Hawaii, all races³²; (18)=Soviet Union, Armenia²⁴; (19)=India³³; (20)=Panama⁹; (21)=Surinam²⁰; (22)=Costa Rica²⁹; (23)=Trinidad^{9,17}; (24)=Jamaica^{9,17}; (25)=Peru, Indians and Mestizos⁹; (26)=Singapore¹⁷; (27)=Malaysia, Kuala Lumpur⁷; (28)=Hawaii, Japanese³²; (29)=Japan, rural¹⁷. In localities 9, 11, 12, 15, 16, 20, 22, 23, 26 and 27 ethnic composition was calculated from ref. 5 as the exact ethnic composition of the population tested for rubella was not stated.

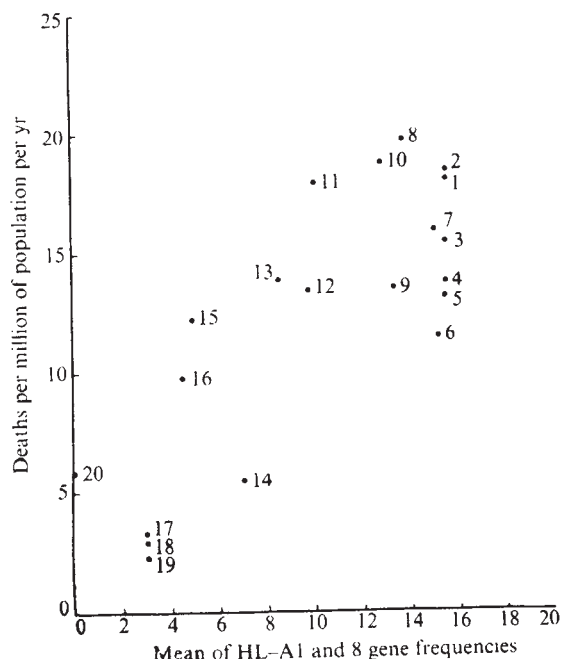


Fig. 2 Geographical correlation between the mean of HL-A1 and 8 gene frequencies HL-A gene frequencies from ref. 4 except where second references (19, 21, 23, 37) are cited, and deaths from Hodgkin's disease (all ages) per million of population per yr. Correlation coefficient (r) = 0.79, t test on r (t_r) = 5.47, P < 0.001. Localities indicated in Fig. 2, together with references for the source of the Hodgkin's disease data, are as follows: (1)=United States, caucasian³; (2)=England and Wales³⁵; (3)=Norway^{3,19}; (4)=Finland^{3,19}; (5)=Sweden^{3,19}; (6)=Australia³; (7)=Canada³; (8)=Holland^{3,21}; (9)=Germany^{3,23}; (10)=Denmark^{3,19}; (11)=Italy³; (12)=India, Bombay, Parsi³⁵; (13)=France³; (14)=India, Bombay, non-Parsi³⁵; (15)=United States, negro^{36,37}; (16)=Jamaica³⁸; (17)=Uganda³⁹; (18)=Kenya⁴⁰; (19)=Nigeria⁴¹; (20)=Japan⁴.

composition. (These were calculated from the Joint Report of the Fifth Histocompatibility Workshop, 1972 (ref. 4). It was sometimes necessary to calculate a mean gene frequency using the percentage of different ethnic groups in a particular population⁶.)

Linear regression analyses on the data for HL-A1 gave a correlation coefficient (r) of 0.72; t test on r (t_r) was 5.31 and the probability (P) was < 0.001. For HL-A8, r was 0.61, t_r was 3.94, P < 0.001; and for the mean gene frequency of HL-A1 and 8, r was 0.68, t_r was 4.78, P < 0.001. These high positive correlations indicate that the frequencies of HL-A1 and 8 are an important factor in the worldwide epidemiology of rubella.

By studying an epidemic of rubella, Hattis *et al.*⁸ have shown that there is a small number of infected individuals who have a high potential for spreading the virus ('spreaders') but that the majority of infected individuals have only minimal potential for spread. We postulate that these spreaders who infect most susceptibles with whom they come in contact are those possessing the HL-A1, 8 haplotype. The mean gene frequency of HL-A1 and 8 in a population would then be related to the number of potential spreaders, and consequently the extent and rate of transmission of virus in that population.

In the past, inexplicable differences in the mode of spread of rubella virus in various populations have been recorded. Statements concerning the spread in Hawaii⁹, Malaysia⁷, Taiwan⁸, Jamaica and Trinidad⁹, where the HL-A1 and 8 gene frequencies are low, may be related to the present hypothesis. In all these areas there are fewer epidemics of rubella than expected, and in some the virus fails to become endemic in spite of high population densities. This would seem to be due

to the low HL-A1 and 8 gene frequencies causing sparser distribution of spreaders in a population which is genetically less susceptible to infection. Although the occurrence of epidemics in these communities will also depend on the interaction of other factors such as opportunities for entry of the virus, the density of the population, and the level of herd immunity at the time of viral introduction, we propose that the most important factor is the frequency of the genes for HL-A1 and 8.

Like that of rubella, the incidence of Hodgkin's disease varies in different ethnic groups; an infectious aetiological agent has been suggested, since the disease occurs in clusters¹⁰, and a single contact can apparently give rise to multiple cases¹¹. Various studies in caucasians with Hodgkin's disease have revealed raised levels of several HL-A antigens¹², such as HL-A1 and 8, W5 and W18, all of which are absent from the Japanese⁴, who have a later onset and slower rise of incidence of the disease¹³. There is a significant increase in the incidence in Japanese moving to the United States. In the United States, 71% of Japanese over the age of 50 are recent immigrants and in this older group the death rate from Hodgkin's disease rises sharply to, and even slightly above, the United States caucasian level¹³. This is comparable to a group of non-immune individuals moving from a situation where an infectious disease is of low incidence and they have a low chance of contacting it, to an environment where it is more common and they have a much higher chance of contacting it; in this case the group moves from a population with low (or absent) frequencies of HL-A1 and 8 (Japan) to a population where these antigens are much more frequent (United States).

Table 1 Correlation of % mean gene frequencies of various HL-A antigens in 20 populations with deaths from Hodgkin's disease*

Antigen	<i>r</i>	<i>t_r</i>	<i>P</i>
HL-A1	0.79	5.47	<0.001
HL-A8	0.75	4.81	<0.001
HL-A1, 8	0.79	5.47	<0.001
HL-A3, 7	0.69	4.04	<0.001†
HL-A2, 12	0.39	1.79	Not significant
HL-A2, 7	0.54	2.72	" "
W5	0.31	1.41	" "
W18	0.39	1.48	" "

* Deaths at all ages per million in that population per yr.

† Populations which are predominantly caucasoid have high levels of HL-A antigens 3 and 7, and these antigens were more frequent (total % gene frequency 240) in our 20 test populations than HL-A antigens 1 and 8 (total % gene frequency 200). Thus the more significant association of HL-A antigens 1 and 8 ($r = 0.79$) with the incidence of Hodgkin's disease (compare 3, 7: $r = 0.69$) indicates that this relationship with HL-A1 and 8 is not simply due to a caucasoid trait not associated with HL-A.

In order to determine whether HL-A1 and 8 played a part in the epidemiology of Hodgkin's disease similar to that found in rubella, data on the incidence of Hodgkin's disease in other ethnic groups were correlated with the mean gene frequency of HL-A1 and 8 (Fig. 2). Linear regression analysis on the data for HL-A1 and HL-A8 produced high correlation coefficients (Table 1). The correlations with W5 and W18 were not significant. We note that the high level of correlation with HL-A1 and 8 does not preclude association of Hodgkin's disease with some of the other HL-A antigens which also vary with ethnicity. Populations which are predominantly caucasian have high levels of HL-A antigens 3 and 7 but the more significant association of HL-A1 and 8 with incidence of Hodgkin's disease indicated that this correlation with HL-A1, 8 was not simply due to a non-HL-A associated caucasoid trait.

These similarities in the epidemiology of Hodgkin's disease and rubella support the concept that Hodgkin's disease has an infectious component, with the racial distribution of HL-A antigens controlling the degree of susceptibility of the population and the number of spreaders¹¹. Possibly there are other

diseases of uncertain aetiology where an infectious agent has been implicated and where the incidence varies with ethnicity (for example multiple sclerosis¹⁴) in which there is a similar epidemiological pattern.

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IgD on cell membranes of human lymphoid cell lines with multiple immunoglobulin classes

IMMUNOGLOBULIN D (IgD) is present in minute amounts in the serum and the biological role of this immunoglobulin class is unknown¹. The finding that a high proportion of lymphocytes bear IgD on their surface membrane² has triggered renewed interest in this molecule.

IgD constitutes only 0.2% of serum immunoglobulins, but approximately 20% of circulating 'bursal' (B) lymphocytes bear surface IgD (ref. 2). In the newborn, serum IgD is usually undetectable and IgD seems to be the predominant class of surface Ig found on B lymphocytes (approximately 60%)³. These findings suggest that cell-associated IgD has an important biological function. Although cell surface Ig is normally restricted to a single class, individual lymphocytes with surface IgD can bear immunoglobulins of other classes as well. Thus, IgM has been found together with IgD in the membrane of peripheral blood lymphocytes of both adults and neonates^{4,5} and on cells of patients with chronic lymphocytic leukaemia⁶; in patients with Sjögren's syndrome the B lymphocytes seem to have IgG, IgA, and IgM on their surfaces in addition to IgD (ref. 7). Critical to the understanding of the significance of IgD, as well as the significance of multiple Ig classes on cell membranes, is whether these immunoglobulins are produced by the cells bearing them, or whether they represent cytophilic molecules or autoantibodies.

Here we report that IgD can be found on all the cells of a relatively high proportion of established human lymphoid cell lines grown in continuous *in vitro* culture. In addition, these IgD bearing cells were found to bear IgG, IgM and IgA. These findings provide direct evidence that lymphocytes produce the IgD found on their surface membranes and that individual lymphocytes can produce as many as four different surface Ig classes.

Cell surface Ig was demonstrated by staining the membranes of living cells with specific fluorochrome conjugated antisera. Of 25 cell lines tested, six were found to bear IgD (Table 1). Cell line 689 stained very brightly for IgD and all the cells in the culture were positive. In other cell lines the staining was less intense and although all the cells had surface IgD, the intensity of staining varied. Those cell lines found to bear δ (IgD) heavy (H) chains were examined for the presence of other H-chain classes in the membrane. In each case all the cells had surface γ (IgG), μ (IgM) and α (IgA) but not ϵ (IgE) H chains, and only one type of light (L) chain, K or λ . Several of the IgD negative cell lines were also found to bear more than one class of surface immunoglobulin. Because of this unexpected finding, multiple staining was confirmed using independently prepared purified antibodies, and precautions were taken to rule out nonspecific staining. The Fc receptor^{10,11} found on B cells could bind spontaneously aggregated γ globulin present in the fluorochrome conjugated antisera. Removal of aggregates, however, by ultracentrifugation at 100,000g for 2 h did not affect the staining results. Non specific binding by the Fc portion of the fluoresceinated antibody to this receptor is unlikely as some fluorescent antibodies, for example, anti-IgE, did not stain the cells. This possibility was excluded when identical staining was obtained with the F(ab')₂ fragment of anti-IgG antibody, which lacks Fc determinants⁸.

The state of these various H chains in the membrane was investigated. Membrane surface molecules can be aggregated by cross linkage with specific divalent antibodies¹². When stained, these can be seen as fluorescent patches which are then actively transported to one pole of the cell to form a cap. Cells of cell line 698 were stained with fluorescein conjugated antibody to K L chain, washed, and incubated at 37°C for 1-3 h to allow capping to

occur. Cells were then incubated with rhodamine-conjugated antiserum with specificity for an H-chain class. This second incubation was performed at 4°C in the presence of sodium azide (0.1%), conditions which prevent capping. Capping induced by antibody to the single L-chain type caused each H-chain determinant to cap along with the L chain. That is, the antiserum directed against each H chain stained only the cap. This indicates that δ , γ , μ and α H chains are each associated with an L chain on these cells and probably exist as discrete Ig molecules. Purified antibodies directed against H chains were less effective in producing caps. Anti- μ and anti- δ reagents induced caps in approximately 30% of cells of the 698 cell line. In these instances repeat staining with antibody to the same H chain stained only the cap and antibodies directed against each of the other H chain classes stained the cell diffusely. Thus μ and δ chains each capped independently of the other three H chains. This is similar to the finding that μ and δ chains cap independently of each other on peripheral blood lymphocytes^{4,5}. Antibodies to γ and α chains stained the cells less intensely and did not produce distinct cap formation.

The cell lines are grown in continuous long term culture in the absence of exogenous human immunoglobulins. It was important to rule out the possibility that the observed staining was due to a few cells in the culture secreting IgD or other immunoglobulins, which then adhered to the remaining cells because of cytophilic properties. Human lymphoid cell lines can secrete more than one Ig class, but secreted IgD has not been found¹³.

Cell line 698, which had the most intense staining for each of these surface Ig classes, was tested for immunoglobulin secretion. The cells were incubated with ¹⁴C-leucine and the incubation medium then analysed for newly synthesised and secreted Ig by radioimmuno-electrophoresis¹⁴. The cells secreted only IgG-K molecules. This result is consistent with previous reports that membrane-bound Ig and secreted Ig are regulated by separate mechanisms¹⁵.

These findings provide direct evidence that surface IgD is synthesised by the cell that bears it and is not merely adherent because of cytophilic properties^{4,6}. Furthermore, it seems that individual lymphocytes have the capacity to produce four different surface Ig classes (IgD, IgG, IgA and IgM). The evidence of Litwin *et al.*¹⁶ that lymphoid cell lines have both μ and γ chains on their surfaces is consistent with these findings. The significance of multiple Ig classes on single lymphocytes is not clear. Although it is tempting to speculate that a switch of surface $\delta \rightarrow \mu \rightarrow \gamma \rightarrow \alpha$ L chains occurs during the ontogeny of the lymphocyte⁴, similar to the switch occurring from μ to γ with secreted immunoglobulins¹⁷, the distribution of multiple H chains found in these cell lines does not support such a hypothesis. No cells lines were found to bear δ or even δ plus μ chains exclusively. Indeed, all the δ bearing lines also bore μ , γ and α chain. We expect that these different Ig classes will have identical L chains and will share identical H chain variable regions as reported for myelomas secreting two different Ig classes¹⁷. Each class may be involved in triggering a specific lymphocyte function. The predominance of IgD bearing cells in the neonatal period suggests that this class of molecules may be involved in the induction of immunologic tolerance⁴. The existence of cell lines in which every lymphocyte bears both IgD and multiple other Ig classes provides a powerful tool for the *in vitro* investigation of these questions.

In the normal adult, differentiation has resulted in restricted expression of surface Ig classes. Multiple H chain determinants, however, have been found on individual lymphocytes in some patients with chronic lymphatic leukaemia¹⁸⁻²⁰ and also in patients with Sjögren's syndrome, an autoimmune disease in which there is a spectrum of benign to malignant lymphoproliferation⁷. These abnormali-

Table 1 IgD bearing cell lines

Cell line	Source	Membrane immunoglobulin					
		IgD	IgG	IgA	IgM	κ	λ
PG-B-1	Normal adult	+	+	+	+	—	+
MBK-1	Infect. mono.*	+	+	+	+	+	—
698	Lymphoma	+	+	+	+	+	—
Cat-B	Hypogammaglob.*	+	+	+	+	+	—
Pt3L3	Xerod. pigment*	+	+	+	+	+	—
Pt3L4	Xerod. pigment*	+	+	+	+	+	—
Molt-4	Acute leukaemia	—	—	—	—	—	—
8866	Acute leukaemia	—	+	—	—	+	—
4265	Acute leukaemia	—	+	+	—	+	—
SB	Acute leukaemia	—	—	—	+	+	—
IM-1	Lymphoma	—	—	+	+	+	—
IM-9	Multiple myeloma	—	+	+	—	+	—
Raji	Burkitt lymphoma	—	—	—	+	+	—
EB-3	Burkitt lymphoma	—	—	—	+	+	—
Maku	Burkitt lymphoma	—	nt	nt	nt	+	—
BSO-B1	Burkitt lymphoma	—	+	+	+	+	—
BFK-B-1	Burkitt lymphoma	—	+	+	+	+	+
Kaplan	Infect. mono.	—	nt	nt	nt	+	—
MBK-B-1	Infect. mono.	—	nt	nt	nt	+	—
Fl-B-1	Foetal liver	—	—	+	+	—	+
Fl-B-4	Foetal liver	—	nt	nt	nt	+	—
Fl-B-6	Foetal liver	—	—	+	+	—	+
NC-37	Normal adult	—	—	—	+	+	—
PG-1	Normal adult	—	nt	nt	nt	+	—
GK-B-1	Normal adult	—	+	+	+	+	+

The cells in these cultures grow in suspension and are fed twice weekly with RPMI 1640 medium (NIH Media Unit) supplemented with 10% foetal calf serum (FCS) (Grand Island Biological NY). These cell lines have undergone a minimum of sixty subcultures. Monospecific antisera to IgG, IgA and IgM were prepared as previously described^{2,7} using insoluble immunoabsorbents. Special precautions were taken to ensure specificity of anti-IgD (refs. 2 and 7). The antiserum was sequentially absorbed with Sepharose coupled to IgG, IgA, IgM, free L chains, and serum from a subject with low IgD levels. No blocking occurred when the cells preincubated with non-fluorescein labelled anti-IgM or anti-IgG were tested for staining with fluorescein-conjugated anti-IgD. When an antiserum specific for IgD (obtained by immunisation of a goat with IgD- λ myeloma protein) was absorbed with a different IgD preparation (IgD- κ) coupled to Sepharose, or with Sepharose-IgA- κ , only the IgD absorbed preparation had a markedly diminished staining capacity. For capping experiments, purified antibody to IgD was prepared by absorption and elution from purified IgD-Sepharose⁸. Antibody to κ chains was prepared using Sepharose-IgG- κ and antibody to λ chains prepared using Sepharose-IgE- λ . Multiple H-chain staining was confirmed using independently prepared purified antibodies. The antisera or antibodies were conjugated with fluorescein or tetramethyl-rhodamine isothiocyanate⁹. The fluorescein or rhodamine to protein (F/P) molar ratio of the reagents used ranged from 1.1 to 2.8:1. Viable cells were centrifuged and the pellet containing 2×10^6 cells incubated with 0.1 ml of undiluted fluorescein or rhodamine conjugated antiserum at 4°C for 30 min. Sodium azide was added when it was necessary to prevent capping. The cells were washed and suspended in medium (RPMI 1640 medium containing 5% FCS) on slides with vaseline-sealed coverslips. The cells were examined by means of a Leitz Ortholux microscope with an Osram HBO 200 mercury arc lamp and a Leitz vertical illuminator.

* Infectious mononucleosis, Hypogammaglobulinaemia, Xeroderma pigmentosum. IgD bearing cell lines were tested for surface IgE and were found to be negative; nt, not tested.

ties may represent dedifferentiation of the malignant or abnormal cells which permits the expression of multiple Ig classes in the membrane in a manner similar to that seen in human lymphoid cell lines. On the basis of the findings in this report cytophilic molecules or autoantibodies need not be invoked to explain the finding of multiple Ig classes in lymphocyte membranes in these clinical states.

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Marrow environment not required for differentiation of B lymphocytes

IN birds, B lymphocytes differentiate from primitive progenitors in the Bursa of Fabricius, which is thought to provide a unique inductive stimulus, as the thymus in both birds and mammals is thought to provide a stimulus for the differentiation of T lymphocytes. A bursal equivalent has not, however, been identified in mammals. Two proposed sites are the bone marrow¹ and the gut-associated lymphoid tissue². We now have evidence, however, that the bone marrow is not an essential site for the differentiation of B lymphocytes in mammals.

B lymphocytes differentiate from pluripotent stem cells in the bone marrow^{3,4}. These cells are probably identical or closely related to haemopoietic stem cells. Specificity-restricted precursors of B cells (PB cells) are also found in bone marrow and seem to represent a stage intermediate between stem cells and functional B lymphocytes^{5,7}. PB cells have immunoglobulin (Ig) receptors but cannot initiate immune responses without further differentiation. Thus, the differentiation pathway of B lymphocytes can be subdivided into at least two stages: the differentiation of PB cells from stem cells, and the production of B cells from PB cells. We have investigated the relative role of the bone marrow environment on these two stages.

Table 1 CFU-S survival in femur from ^{89}Sr -treated donors

Time at 4° C (h)	Donor	Cells per femur ($\times 10^6$)	CFU-S per femur*	% Survival*
0	Control	180	3,700 $\pm$ 530	13 $\pm$ 6.0
	^{89}Sr -treated	110	490 $\pm$ 210	
24	Control	220	1,800 $\pm$ 550	10 $\pm$ 3.4
	^{89}Sr -treated	130	180 $\pm$ 29	
48	Control	180	1,500 $\pm$ 200	0.80 $\pm$ 0.16
	^{89}Sr -treated	68	12 $\pm$ 1.7	

Mice (F_1 hybrids between C57BL/6 and C3H) received an intraperitoneal injection of 50 μCi ^{89}Sr . Twenty-four hours later the femurs were removed from fifteen of these mice and from fifteen normal mice. These femurs were placed in a Petri dish on filter paper moistened with phosphate-buffered saline and stored at 4° C. At the times indicated ten femurs were taken from each group and used to prepare marrow cell suspensions. Aliquots were injected into irradiated (950 rad) recipients to measure CFU-S.

* Mean values $\pm$ one s.e.m.

It is possible by injection of radioactive strontium (^{89}Sr), to make the marrow a hostile environment for differentiation^{8,9}. This isotope is taken up exclusively by bone. When ^{89}Sr decays, it emits 1.5 MeV electrons and irradiates the bone marrow cavity with doses of radiation that inhibit proliferating cells⁸. Because the energy of the radiation is insufficient to penetrate bone and irradiate other organs in the animal, it is possible by this means to irradiate specifically and continuously the entire bone marrow of an animal without affecting the integrity of other organs.

We first estimated how rapidly cells in bone marrow were inactivated by an intraperitoneal injection of ^{89}Sr . Twenty-four hours after injection, the intact femurs were taken from these mice and from normal mice and stored at 4° C. After various times, the marrow was collected from these femurs and the number of haemopoietic stem cells per femur was determined by injection of the cells into irradiated recipients. These stem cells form easily detectable colonies in the spleens of irradiated mice, and enumeration of the number of spleen colony-forming units (CFU-S) provides a quantitative estimate of the number of surviving haemopoietic stem cells^{10,11}. Because the radiation sensitivity of CFU-S and B cells is similar¹², the survival of CFU-S in ^{89}Sr -treated recipients should be similar to that of B cells and their precursors located in marrow. These measure-

ments (Table 1) suggest that CFU-S are killed exponentially with time and have a halflife of approximately 10 h in a marrow environment irradiated by ^{89}Sr . In four other experiments in which CFU-S survival was measured 4 d after ^{89}Sr treatment, survival was always about 1% relative to untreated controls.

On the basis of this short halflife for a known stem cell in ^{89}Sr -treated recipients, we attempted to measure the function of mature B cells, PB cells and lymphoid stem cells in mice with defective marrow. Recipient mice received intraperitoneal injections of 50 μCi of ^{89}Sr . Four days later, both these mice and untreated controls received whole body irradiation from a ^{137}Cs irradiator with doses ranging from 750 to 950 rad and were used to measure B cell, PB cell or stem cell activity as indicated below.

To test for loss of B cell activity, each recipient received 2×10^7 spleen cells from normal donors, together with 5×10^8 sheep red blood cells (SRBC). Eight days later, they were killed and antibody-producing cells were counted per spleen using the Jerne plaque-forming cell (PFC) assay¹³, to give a measure of B-cell activity in these recipients. Table 2 shows that in three experiments, B-cell activity was not affected by pretreatment of recipients with ^{89}Sr ; if anything, the response was greater in pretreated recipients than in controls.

Table 2 Function of lymphoid and haemopoietic cells in ^{89}Sr -treated mice

Assay	Experiment no.	Response int†		^{89}Sr -treated $\times 100$ ‡ control
		Control recipient	^{89}Sr -treated recipient	
B cell	1	703 PFC per spleen	987 PFC per spleen	140 (112-176)
	2	629 PFC per spleen	1,803 PFC per spleen	286 (185-442)
	3	1,504 PFC per spleen	1,196 PFC per spleen	79 (51-122)
PB cell	1	1,278 PFC per spleen	730 PFC per spleen	57 (34-96)
	2	1,606 PFC per spleen	899 PFC per spleen	56 (31-102)
	3	6,792 PFC per spleen	1,993 PFC per spleen	29 (20-42)
Stem cell	1 (25)*	22,600 PFC per spleen	14,600 PFC per spleen	65 (38-109)
	2 (24)	25,300 PFC per spleen	11,100 PFC per spleen	44 (35-55)
	3 (33)	26,000 PFC per spleen	15,600 PFC per spleen	60 (41-88)
CFU-S in marrow	1 (25)	636 CFU-S per femur	35 CFU-S per femur	5.5 (4.8-6.2)
	2 (24)	860 CFU-S per femur	16 CFU-S per femur	1.9 (1.3-2.5)
	3 (33)	1,206 CFU-S per femur	52 CFU-S per femur	4.3 (2.8-5.8)
CFU-S in spleen	1 (25)	783 CFU-S per spleen	1,933 CFU-S per spleen	247 (197-297)
	2 (24)	1,500 CFU-S per spleen	2,800 CFU-S per spleen	187 (159-215)
	3 (33)	1,512 CFU-S per spleen	2,889 CFU-S per spleen	191 (149-233)

In experiments 1 and 2 the primary recipients received 750 rad whole body irradiation; in experiment 3, 900 rad.

* The numbers in parentheses are the days after transplantation, that is, in experiment 1, the stem cell and CFU-S assays were done 25 d after transplantation of cells into the irradiated (950 rad) recipients.

† The PFC responses are geometric means of the responses measured in individual spleens. The CFU data are arithmetic means.

‡ The values in parentheses are one standard error above and below the mean.

To measure PB cell activity, bone marrow was separated by velocity sedimentation as described previously^{5,14}. Those cells sedimenting between 4.5 and 8.0 mm h⁻¹ were pooled to provide a suspension enriched for PB cells and stem cells but nearly free of B cells⁶ and 3×10^6 cells from the pool were injected, together with 5×10^7 thymus cells, into recipient mice prepared as above. Seven days later the recipients were immunised with an intraperitoneal injection of 5×10^8 SRBC. Eight days after immunisation, the PFC per spleen were measured for each mouse. Lafleur *et al.*⁵ showed that under these conditions the PFC response is derived primarily from PB cells. Table 2 shows that the differentiation of PB cells is not markedly suppressed in ⁸⁹Sr-treated recipients.

The differentiation of B lymphocytes from cells more primitive than PB cells was also studied according to the method of Lafleur *et al.*⁶. Cells were separated and injected into recipients as described above. Approximately 4 weeks later, recipients were given an intravenous injection of 5×10^7 thymus cells (to ensure that there was no defect in T cell function) and 5×10^8 SRBC. Six days later, at the time of peak response, the PFC response was determined. As with PB cells, there was a slight suppression in the PFC response in ⁸⁹Sr-treated recipients (Table 2).

Radioactive strontium has a half-life of 54 d and should therefore have suppressed the activity of cells in marrow throughout the time period of our experiments. This suppression was confirmed by measurements of CFU-S in the femurs and spleens of the ⁸⁹Sr-treated recipients. When mice were killed for measurement of PFC for the stem cell assay, their spleen and marrow cells were also injected into normal irradiated recipients to count the CFU-S. CFU-S content in the femurs of ⁸⁹Sr-treated recipients was suppressed markedly compared with the control (Table 2). Also, as reported before⁸, the spleens of such recipients had increased numbers of CFU-S. These results show that cells such as CFU-S, which must proliferate to function, do not survive in the marrow during the time required for experiments such as ours.

The CFU-S data clearly show that pretreatment of mice with ⁸⁹Sr effectively destroyed the bone marrow environment as a site for normal differentiation. Nevertheless, the differentiation of PB and stem cells in such recipients was only slightly suppressed. If this differentiation had to occur by proliferation of stem cells in the bone marrow environment, the suppression of activity should have been of the same order as seen for CFU-S in the marrow. That such suppression was not observed is evidence that the marrow environment is not essential for the development of B lymphocytes.

There are several possible explanations for the small suppression observed for the PB cells and stem cells assayed in ⁸⁹Sr-treated recipients. Perhaps the most likely is that it is a result of the ability of many B cells to circulate through the lymphoid system¹⁵. Because of this property, it is reasonable to expect that some B cells will be inactivated as they migrate through the bone marrow. In support of this suggestion is our observation that the B cell activity in spleen decreases to approximately 30% of normal within 4 d after injection of 50 μ Ci of ⁸⁹Sr (unpublished data of R.A.P.).

An alternative explanation of the suppression of PB and stem cell activity is that the differentiation of functional B cells requires a transient exposure to elements in bone marrow. If the radiation sensitivity of B cell precursors is the same as for B cells and CFU-S, a suppression of 50% would be consistent with a 10-h exposure to the marrow environment. This 10-h estimate is an upper limit and the data are consistent with there being no required residence time in marrow.

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Oestrogen receptors and antagonism of steroid hormone action

NON-STEROIDAL anti-oestrogens antagonise the effects of oestrogen on uterine growth, vaginal cornification and ovulation¹, and their mechanism of action has been suggested to reside in their ability to compete for cytoplasmic oestrogen binding sites, or receptors, thereby reducing the formation of receptor oestrogen complexes²⁻⁴. Since the formation of these complexes in oestrogen target tissues, with subsequent translocation to nuclear sites, is considered the primary event in oestrogen action^{5,6}, the reduction in their number would lead to decreased physiological responses to oestrogen. This implies that the receptor antagonist complex should have a lower intrinsic biological activity than that of the receptor oestradiol complex; that is, the ability of the receptor antagonist complex to stimulate oestrogenic responses would be less than that of the receptor oestradiol complex. Based on these assumptions, oestrogen antagonism should be observed after a single injection of oestrogen and antagonists; we have now found that this is not the case. Thus, non-steroidal anti-oestrogens could not be acting as antagonists by competing initially for oestrogen receptors.

Immature rats, 21–22 d old, were injected with 2.5 μ g oestradiol, 100 μ g nafoxidine hydrochloride (Upjohn 11,100A 1-(2-[P-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]pyrrolidine hydrochloride) or a combination of the two, and uterine dry weights were determined 24–48 h later. The increases in uterine dry weight after a single injection of oestradiol, nafoxidine or oestradiol plus nafoxidine were identical 24 h after injection. By 48 h nafoxidine or oestradiol plus nafoxidine was more effective than with oestradiol alone in stimulating uterine growth (Fig. 1). Therefore, after

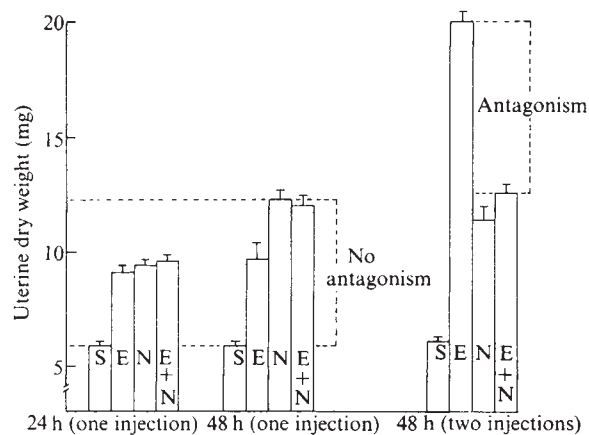


Fig. 1 Agonistic and antagonistic effects of nafoxidine on uterine growth. Immature female rats were injected with saline (S), 2.5 µg of oestradiol (E), 100 µg of nafoxidine (N) or oestradiol plus nafoxidine (E + N) according to the following design. Two groups received one injection and uterine weights were determined 24 or 48 h later. A third group received two injections 24 h apart and uterine weights were determined 24 h after the second injection. Each value represents the mean $\pm$ s.e.m. for three or four determinations with five or six animals per determination.

a single injection of oestradiol plus nafoxidine there was no antagonism; indeed, nafoxidine clearly acted as an oestrogen. The antagonistic properties of nafoxidine could be observed, however, when the compounds were administered at 24 h intervals and the uterine weights were determined after 48 h (Fig. 1). Serial injections (between three and eight) at 24 h intervals have often been used routinely and it is well established that oestrogen antagonism can be observed under these conditions (refs 1 and 7-9 and Fig. 1, two injections). Such routine methodology has resulted in the failure to detect a lack of antagonism after a single injection. It should be noted that we have since used much smaller doses of antagonists than were injected in the work described with similar results; that is, smaller doses, 1-10 µg have both agonistic and antagonistic properties (to be published elsewhere).

We have proposed that anti-oestrogens antagonise oestrogen-induced uterine growth as a result of their failure to stimulate the replenishment of the cytoplasmic oestrogen receptor¹⁰. That is, after cytoplasmic binding and nuclear translocation of receptor antagonist complexes, no additional receptor is provided to the cytoplasmic pool. This failure to stimulate the replenishment of cytoplasmic receptor renders the uterus insensitive to subsequent injection of oestrogen and therefore antagonism can be observed.

To test this hypothesis further and to establish the general nature of this phenomenon, we have examined the effects of two other oestrogen antagonists, CI-628 and (α -[4-pyrrolidinoethoxy]phenyl-4-methoxy- α -nitrostilbene) and

clomiphene (Triethylamine, 2-(P-[chloro-1,2diphenylvinyl]-phenoxy)-citrate). These compounds, as well as nafoxidine, were administered either alone or in combination with oestradiol, and the uterine weight and cytoplasmic oestrogen receptor content were determined. The concentration of cytoplasmic receptor was determined by the ³H-oestradiol exchange-charcoal adsorption method^{10,11} which facilitates evaluation of total cytoplasmic receptor and avoids complications arising from assays that measure only free or unfilled receptor sites¹². Bound nafoxidine, CI-628 and clomiphene (our unpublished results) are freely exchangeable with ³H-oestradiol in this assay procedure^{10,11}.

As Table 1 shows, all oestrogen antagonists examined were clearly uterotrophic and not antagonistic when given in combination with oestrogen. Thus, CI-628 and clomiphene are similar to nafoxidine in this regard. In addition, all three antagonists, alone or in combination with oestradiol, reduced the quantity of cytoplasmic receptor 24 h after treatment (Fig. 2). Thus none of these antagonists could stimulate the replenishment of the cytoplasmic receptor, whereas oestradiol treatment resulted in a significant increase in receptors (Fig. 2). Therefore, the ability of the uterus to respond to oestradiol 24 h after treatment with an antagonist would have been greatly reduced. This was clearly the case with nafoxidine, after which subsequent oestradiol treatment induced no uterine growth

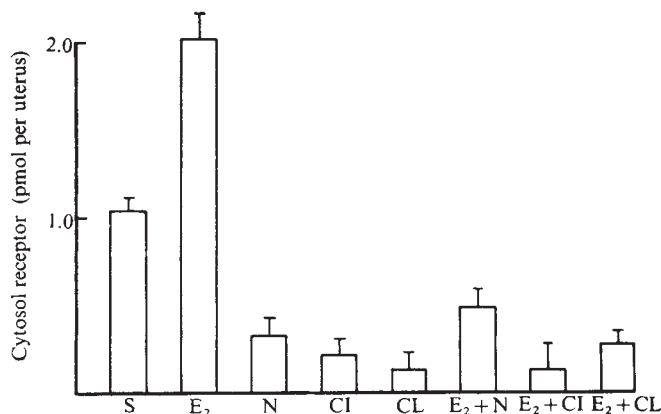


Fig. 2 Effect of oestradiol and oestrogen antagonists on the quantity of cytoplasmic receptor 24 h after treatment. Immature female rats were injected with 2.5 µg of oestradiol (E), 100 µg of nafoxidine (N), 0.5 mg of CI-628 (CI), 10.0 mg of clomiphene (CL) or a combination of oestradiol plus the antagonists. The quantity of total cytoplasmic receptor was determined 24 h after treatment by the ³H-oestradiol exchange-charcoal adsorption procedure (see text). Each value represents the mean of three or four experiments with five or six animals per experiment.

Table 1 The effect of oestradiol and oestrogen antagonists on uterine growth after a single injection

Treatment	Uterine wet weight (mg)	
	24 h	48 h
Saline	36.8 $\pm$ 1.5	40.5 $\pm$ 2.3
Oestradiol (2.5 µg)	53.4 $\pm$ 1.0	60.8 $\pm$ 2.7
Nafoxidine (100 µg)	54.2 $\pm$ 2.0	60.3 $\pm$ 4.2
CI-628 (500 µg)	51.2 $\pm$ 2.1	62.0 $\pm$ 3.6
Clomiphene (10 mg)	50.1 $\pm$ 2.8	69.0 $\pm$ 4.5
Oestradiol + nafoxidine (2.5 + 100 µg)	54.5 $\pm$ 1.9	63.2 $\pm$ 3.9
Oestradiol + CI-628 (2.5 + 500 µg)	52.9 $\pm$ 2.3	72.3 $\pm$ 5.7
Oestradiol + clomiphene (2.5 + 10 mg)	50.3 $\pm$ 2.8	74.0 $\pm$ 6.8

Immature female rats were injected with the compounds indicated and the wet weight of their uteri was determined 24 or 48 h later. All values represent the mean $\pm$ the s.e.m.

(Fig. 1). In contrast, the quantity of cytoplasmic receptor in animals pretreated with oestradiol was twice that of the controls and the uterus was very responsive to a second injection of oestrogen (Fig. 2)¹⁰. Thus these compounds are both agonistic and antagonistic. The anti-oestrogens are agonists because they stimulate the metabolic and regulatory pathways that cause uterine growth. They are also antagonists, however, because they fail to stimulate the replenishment of the cytoplasmic receptor and thus leave the uterus insensitive to oestrogen.

A further factor that might contribute to oestrogen antagonism is that anti-oestrogens might stimulate cellular hypertrophy, thereby increasing uterine weight, but fail to stimulate uterine hyperplasia. In such a case the number of cells able to respond to subsequent oestrogen treatment would be reduced and this reduction would result in diminished uterotrophic responses than when oestrogen alone was used. To examine this possibility and to characterise further the growth induced by the anti-oestrogens, we have mea-

sured the quantity of DNA and protein in the uterus at various times after the administration of hormone or antagonist. Both oestradiol and nafoxidine increased DNA content within 48 h; thus there was no apparent difference in their capacity to elicit uterine hyperplasia (Fig. 3). Increased mitotic activity has also been observed to occur with either compound (unpublished results). The obvious difference between the uterotrophic responses elicited by the two compounds was the sustained stimulation of protein synthesis observed with nafoxidine (Fig. 3). Apparently the long term uterotrophic effects of nafoxidine arise from the ability of the receptor antagonist complex to stimulate cellular hypertrophy. These experiments indicate that oestrogen antagonism is not due to an inability of nafoxidine to stimulate cell growth. In fact, it was more effective than oestradiol when uterotrophic responses were compared 72 h after treatment (Fig. 3).

These studies are not sufficient to determine whether receptor replenishment is the only uterotrophic response not stimulated by oestrogen antagonists. We feel, however, that most response pathways are stimulated since the increases in uterine protein, DNA and weight induced by the antagonists are equal to those induced by oestradiol. Our findings are important in the interpretation of experiments in which oestrogen antagonists have been used to block ovulation, implantation and other oestrogen-stimulated responses. In these systems oestrogen antagonists may stimulate oestrogenic responses just as in the uterus and, in a similar fashion, may not stimulate the replenishment of cytoplasmic receptor. If this is true, the agonistic nature of the anti-oestrogens as well as their inability to promote the replenishment of the cytoplasmic receptor, and its subsequent binding and interaction with oestrogen, would become important considerations in the interpretation of such systems.

Our investigation has important implications with respect to the mechanism of action of non-steroidal antagonists

of oestrogenic hormones. Theories and model systems designed to describe the action of oestrogen antagonists must include more than a simple competition for steroid binding sites. It has been assumed that the dissociation constant, K_d , for the binding of antagonist to steroid receptors is related directly to its capacity to block steroid activity; that is, the K_d is that concentration of antagonists that will block or antagonise 50% of the maximal (physiological) biological response. Assays for the action of oestrogen antagonists, however, usually have involved serial injections. The marked depletion of cytoplasmic receptor under such conditions would invalidate the model because implicit in it is the assumption that receptor is present at normal levels during each period of treatment.

In conclusion, we believe that non-steroidal anti-oestrogens are antagonists not because they compete for cytoplasmic oestrogen receptor to produce a complex which is a poor stimulator of growth, but because they fail to stimulate the replenishment of the receptor. Thus the uterus of the antagonist-treated animal contains relatively few cytoplasmic receptors and is insensitive to oestrogen, whereas the uterus of the oestrogen-treated animal contains many cytoplasmic receptors and is highly sensitive to oestrogen.

This work was supported by grants from the National Institutes of Health and the American Cancer Society. We thank Dr P. W. O'Connell, Upjohn Co.; Dr Jerry Reel, Parke-Davis and Co. and Mr A. Richardson, jun., Merrell-National Laboratories for the nafoxidine, CI-628 and clomiphene. We also thank L. Hseuh for technical assistance.

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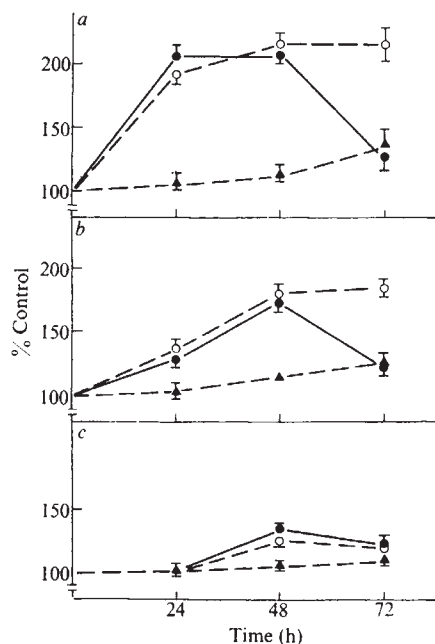


Fig. 3 Effect of oestradiol and nafoxidine on uterine protein and DNA after a single injection. Immature rats were injected with 2.5 μ g oestradiol (●), 100 μ g of nafoxidine (○) or saline (▲) and the wet weight, protein¹³ and DNA¹⁴ were determined 24, 48 and 72 h later. The control values at the beginning of the experiment were: wet weight, 32.5 $\pm$ 3.1 mg per uterus; protein, 4.5 $\pm$ 0.2 mg per uterus; DNA, 360 $\pm$ 12 μ g per uterus. Each value represents the mean of five or six determinations with three to six animals per determination. a, Wet weight; b, protein; c, DNA.

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reviews

ONCE more Professor Glob puts us in his debt. Five years ago his best seller *The Bog People* was published in English; now we are presented with its sequel—*The Mound People*—a lively and enthralling account of the inhabitants of Denmark at one of the peaks of the country's cultural development in the second millennium BC. In the first part of the book the author sets out to describe a selection of the remarkably preserved burials by which the Bronze Age inhabitants are known. Having thus introduced them to us, down to the last detail of their clothing, he proceeds to reconstruct something of the social economic and religious background of the times.

That so much is known is because of a combination of factors. The bodies were buried, fully clothed, in coffins made from split oak logs which were hollowed out, and buried beneath high mounds of turf and soil—the barrows which still today dominate the skylines in many parts of Denmark. Gradually the rain water percolated through the mound, and laden with tannin leached out of the oak, penetrated the coffins and thus preserved clothing as well as human skin, hair and sometimes brain tissue.

Early discoveries, dating back to the beginning of the nineteenth century,

Bringing the dead to life

The Mound People. By P. V. Glob. Pp. 184+75 plates. (Faber and Faber: London, May 1974.) £4.25.

BARRY CUNLIFFE

were not particularly well observed and conservation techniques were unable to cope with such delicate material. But more recently there have been several notable successes: the most fascinating is undoubtedly the discovery in 1929 of a young girl of 20 or so from Egtved, whose midriff-exposing tunic and see-through cord skirt were greeted with shocked incredulity by certain members of the Danish academic world until it was pointed out that several contemporary bronze figures were shown clad in similar skirts.

There can be very little doubt, from the surplus energy which must have been expended in constructing the great mounds, and from the richness of the personal equipment buried with

the dead, that the coffin burials represent the aristocratic class of bronze age society. Glob estimates that the turf required for a single barrow mound would have stripped between two and four acres of pasture of its soil cover. When it is remembered that many thousands of these barrows were built in a comparatively short period, something of the devastation of the land can be appreciated.

The conspicuous consumption of labour for constructing the mounds and the wealth of the objects of gold and bronze buried within them, metals which incidentally would have had to be imported into Denmark from further south, is a firm reminder of the stability of the native economy, based on the export of local products such as amber, cattle, furs and perhaps slaves, and of the strength of the military hierarchy. All this is discussed in great detail in the final chapters, which bring together a wealth of archaeological facts moulded into a cohesive story of a people and their lives, with Glob's customary mixture of insight and intelligent speculation.

Professor Glob evidently enjoys his archaeology. In this attractively written and beautifully illustrated book he offers us the chance to share his enjoyment. The opportunity is irresistible.

THE number of books on pesticides must by now be big enough to construct a largish pulpit from which to continue the environmental argument over such chemicals in general, and over the chlorinated insecticides, long since cast as the villains of the piece, in particular. To those of us involved in agriculture, particularly tropical agriculture, and public health, the banning of these particular chemicals would constitute a disaster, with world food production the victim; the oil crisis may indeed yet cause such a shortage of pesticides as to bring about an unfortunate involuntary demonstration of their beneficial effects to mankind.

It is therefore a pity that the various prefaces and introductions to this new series on the chemistry of pesticides seem somewhat apologetic and vague as to the reason why it has been launched "at a time that seems to mark the twilight . . . of the chlorinated insecticides". If men are wise,

this twilight will be a long one, because the much heralded "third generation insecticides" will not be a practical economic proposition for many years during which many more million mouths will need filling. But during that time we need to re-appraise the

Necessity for DDT

Chlorinated Insecticides. Vol. 1: Technology and Application. By G. T. Brooks. Pp. 249. (CRC Press: Cleveland, Ohio, 1974) \$30.00.

potential and refine the deployment of chlorinated pesticides and for this a knowledge of their chemistry will be vital. This is adequately and clearly covered in this volume, built up from a very extensive bibliography; included in the account of each group which

comprise the DDT group, the diene group, HCH and Toxaphene, is a comprehensive summary of its syntheses and properties and the principles of analysis.

The weakest parts of the book are the sections dealing with application, which are mainly a rehash of information already published in such standard texts as A. W. A. Browne and J. R. Busvine. This orthodoxy underrates the vital and increasing importance of correct formulation in application, particularly for the chlorinated insecticides, which can increase their efficiency and reduce their environmental danger, and which must be based on a detailed knowledge of their chemistry.

It is difficult to assess the value of the entire book without reference to volume 2, which will deal with biological and environmental effects, but this volume is competent and covers the ground adequately and will be a useful reference work in pesticide laboratories.

P. T. HASKELL

Energy in historical context

Discovery of the Conservation of Energy. By Yehuda Elkana. Pp. x+213. (Hutchinson: London, 1974.) £4.00.

THIS book attempts far more than a straightforward review of the historical development of the concept of energy and of the theory of its conservation. Dr Elkana writes in his preface that "the fundamental idea behind [the book] is the essential unity of all aspects of the intellectual enterprise". Thus he discusses not only the internal issues (which in the case of the energy concept are wide-ranging, extending through all branches of science and over a number of centuries) but also the external issues of personality, social climate, metaphysical background and national influence. The task he sets himself is a valuable one; I. B. Cohen, in his foreword, describes him as breaking new ground in trying to resolve the internalist-externalist debate, and certainly if a synthetic view of the historical development of science is ever to be achieved, such a resolution is essential.

Despite his somewhat grandiose undertaking, Dr Elkana's book is not a long one, and at the end of it the reader is left feeling a little breathless. Many lengthy discussions are curtailed by recourse to the assumption that the reader already has a thorough background knowledge of the extensive literature available, and any aspect of the subject which the author feels has already been adequately covered he omits, giving instead only a very brief critique of secondary sources. This seems well justified in a study of this nature, but unfortunately creates some artificial imbalances for the unwary reader. Thus, for example, Humphrey Davy is allotted as much space as Faraday or Joule, who traditionally play a greater part.

Hermann von Helmholtz is, not surprisingly, the central figure in the book. Dr Elkana puts forward the thesis that the very vagueness of the energy concept prior to Helmholtz's work—a vagueness he illustrates elaborately by examples of the confusions centred on words such as 'energy', 'kraft' and 'force'—was an essential element, which, combined with a metaphysical belief in the conservation of some undefined entity, eventually enabled Helmholtz to reach a formal mathematical statement of a general conservation of energy law in the 1840s. This then allowed the concept of energy itself to be clarified. He emphatically rejects any claims made that Newton, Carnot, Faraday or even Joule had an implicit awareness of the general law before Helmholtz's statement of it. He rejects too some of the factors which have traditionally been considered im-

portant in the history of the law, such as the search for a perpetual motion machine and the development of the steam engine.

The author's tendency to expound his aims and general historical method occasionally becomes distracting, especially since this is not confined to his introduction, but sometimes insinuates itself into discussions of a less general nature. Hence interwoven, sometimes irritatingly, with his thesis about the concept of energy is a second, general thesis on how such concepts ought to be studied. Occasionally, too, he introduces sweeping historical assumptions which take the reader a little by surprise. Nevertheless his approach is very refreshing, and he raises numerous exciting and stimulating questions. On every page there are suggestions for further research—indeed the author leaves almost too many avenues unexplored. The book should certainly provide an excellent general scheme within which further study of the history of the energy concept can be placed.

It is to be regretted that the paper republished as an appendix could not have been incorporated into the main body of the book—or at least printed in type of the same size as that used in the rest of the text.

LAURA TILLING

Logic in plant chemistry

Chemotaxonomy of Flowering Plants. By R. Darnley Gibbs. Vol. 1: Constituents: pp. xx+1,680; vol. 2: families; pp. 681+1,274; vol. 3: orders: pp. 1,275–1,980; vol. 4: bibliography, index: pp. 1,981–2,372. (McGill: Queens University, Montreal and London, June 1974.) \$135 per set.

Chemistry in Botanical Classification. Edited by G. Bendz and J. Santesson. (Proceedings of the 25th Nobel Symposium, August 1973.) Pp. 320. (Academic Press: New York and London, July 1974.) \$27.50; £13.20.

DR GIBBS is the doyen of English-speaking chemotaxonomists; and it is gratifying to receive the four large volumes which he has now produced.

After a rather lengthy introduction, the author settles down in volume 1 to 800 pages of chemical plant constituents, arranged alphabetically under headings ranging from acetylenic compounds at one end to waxes at the other, complete with diagrams of structural formulae. For the botanist, at least, this provides a very useful dictionary; and it continues to the middle of volume 2, when there is a switch to a second dictionary (of 280 pages), this time of the families of the dicotyledons and monocotyledons, also

arranged alphabetically. Each family, old or new, is given a paragraph with nomenclatural details and references, and there is an attempt to define the position of the family in the orders in which leading taxonomists during the last 150 years have placed it. I found these paragraphs too compressed, often confusing, and of limited value.

Dr Gibbs then proceeds to put the chemical and taxonomic information together, to see if it is possible in this way to define more effectively the orders of flowering plants. The twelfth edition of Engler's syllabus is used as a basis for the treatment, which extends over the next 800 pages, to the end of volume 3. Orders which are minor or not generally recognised are dealt with briefly, but under each major order the relevant chemical data are given, together with the author's conclusions as to their bearing on the composition and limits of the order. Thus, under the Tubiflorae, 26 families are described chemically in 40 pages of text and five tables, and this is summed up in a couple of pages of discussion.

The chemical evidence is based partly on published work, and partly on the author's personal tests, which cover a wide range of species. The discussions are usually critical and sometimes illuminating, but it is not always easy for the reader to evaluate for himself the great mass of data presented or to follow the author in his reasoning. Essentially, he is trying to make sense of the patterns of distribution of chemical compounds among large taxa; and without advanced statistical treatment (and with so many gaps in the information) he cannot get very far. But the book will be of value as a storehouse of data and as a record of the author's experience. The fourth volume contains the bibliography, the valuable index and some addenda.

The book of the Nobel symposium is an excellent piece of work. In it, some 30 distinguished specialists present papers on a series of topics, particularly the role of special classes of compounds in the systematics of plant groups of various kinds and sizes, together with critical consideration of the discipline of chemotaxonomy.

The papers are all short and to the point, presenting the special data or individual views of the author concerned, and often controversial; and each is followed by a page or so of discussion. There are also a general discussion and a summing up. The symposium must have been most stimulating to the participants; and this sense of stimulation is communicated in the volume, which is highly recommended.

D. H. VALENTINE

Medical meeting

Medical Research Systems in Europe. Edited by E. P. Woodford. (A joint Wellcome Trust-Ciba Foundation Symposium held in London in March 1973. Ciba Foundation Symposium 21, New Series.) Pp. viii+335. (Elsevier, North-Holland: Amsterdam, London and New York, 1973.) Dfl. 43; \$17.20.

WITH all the turmoil in research circles following the British government's acceptance of the Rothschild "customer/contractor" principle of research funding and organisation, this meeting was very timely. Twenty-two speakers from all the major countries in western and eastern Europe except for the Soviet Union circulated in advance papers describing the situation in their countries. This was a wise step, for though the papers are very useful sources of information, a repetition of the various types of support and organisation would have been tedious. The main purpose of the symposium was discussion, and this comprises some 80 pages of fascinating reading, indicating a real exchange of ideas.

Although the meeting was ostensibly about research, it also dealt fully with medical education and administration, and with the ways in which students could be channelled in to research careers. The most obvious differences between the different countries stemmed from their admission policies to medical schools. Most countries, both western and communist, now restrict their student entry to numbers near to those which the teachers think reasonable—the so-called "*numerus clausus*". Even so there were complaints of overcrowding and difficulties in communication. In Lisbon, where entry is unrestricted, a first-year entry of more than 2,000, with a 50% drop out, was found to be prejudicial to both research and teaching. Several speakers spoke of the danger to the community of the disappointed drop-outs, though this seemed more serious to western than eastern participants. There was acceptance of high numbers of students by C. H. Nachev of Bulgaria, though the proportion receiving maintenance grants there is only 40% of that in Britain.

Nobody was entirely satisfied with the existing situation. Everybody deplored the lack of interest in research by many of the abler students, which seemed more serious than any shortage of funds. In all countries there was some division between work done in universities and separate institutes—where student numbers were high, institutes seemed to play the greatest part. Though the need to cooperate with scientists without clinical qualifications was realised, P. J. Hjort of Norway suggested, not entirely in fun,



An early X-ray photograph of "the bones in the fingers of a living human hand. The third finger has a ring upon it." Described by W. C. Röntgen, writing on "a new kind of rays," in *Nature* in 1896. Reproduced in *Patterns in Physics*. By W. Bolton. Pp. vii+448. (McGraw-Hill: Maidenhead, March 1974.) £3.95.

that, perhaps, "these invaders should be driven back, under the slogan 'medicine for the doctors'". This sounded somewhat outdated to the ears of those from Britain, where the most eminent of the directors of our most prestigious medical research institutes have been, from a medical viewpoint, laymen. Clinicians and others regretted the difficulties of obtaining the cooperation of patients, though H. Berndt said that in East Germany "patients are well educated and understand the value of clinical research, so they are cooperative".

The one thing missing from the symposium was any objective assessment of the success of any particular system of education and research. Experience elsewhere has indicated that there is little correlation between research productivity and available facilities. There was little which suggested that any political system was less—or more—likely to make research particularly productive. R. Saracci of Italy did indeed defend the principle of commissioned research, quoting Peter Medawar's point that painters and musicians in the past produced commissioned masterpieces, so "why shouldn't scientists react similarly?" This book was perhaps of greatest value in providing the background information for a survey of research efficiency, something which has still not been made, even by those who are responsible for the recent drastic changes in research organisation in Britain and elsewhere.

KENNETH MELLANBY

Introduction to superfluids

Superfluidity and Superconductivity. By D. R. and J. Tilley. Pp. viii+262. (Modern University Physics Series.) (Van Nostrand-Reinhold: New York and London, July 1974.) £9.50 cloth; £4.95 paper.

It has long been recognised that the phenomenon of superfluidity in helium four has much in common with that of superconductivity in metals, each being marked by the macroscopic flow of material particles without dissipation of energy. A major defect of all previous introductory texts has been that although the similarities were in many cases commented upon, a genuinely integrated treatment was not attempted: it is this deficiency which the Drs Tilley have set out to rectify.

They start with brief descriptions of the observed properties of superfluid helium and of superconductors. The macroscopic wave function is then introduced on page 32; and it is around this unifying concept that the rest of the book is built, with chapters discussing the two-fluid model of HeII, electrodynamics and tunnelling in superconductors, vortex states, Josephson effects, Ginzburg-Landau theory, and the possibility of superfluidity in other systems.

The level assumes no more than an undergraduate knowledge of statistical mechanics, electricity and magnetism, and solid state physics, and the mathematical demands on the reader are remarkably modest. It is astonishing how much material the authors have managed to cram into the 248 pages of main text: I suspect that the large concentration of conceptual challenges, while highly stimulating to the beginning Ph.D. student, may prove rather strong meat for the majority of M.Sc. students.

A book of this scope is bound to attract minor quibbles from almost every reader whose particular research interest lies among the topics discussed. It does, however, seem rather a pity that, after a lucid exposition of Landau's famous explanation of superfluidity in terms of the elementary excitation spectrum, the authors fail to mention the one and only physical situation where this approach leads to an accurate numerical value of the critical velocity, namely, the passage of negative ions through pressurised HeII. On the other hand, I very much like the chapter on vortex states, a topic which lends itself particularly well to a unified treatment; and the section which presents a point by point comparison of vortices in superconductors with those in superfluid helium, emphasising the differences as well as the similarities, will be especially useful to novitiates.

A measure of the continuing vigour of research in this field is given by the extent to which the book has been overtaken by important new discoveries during its writing. The most significant of these came with the observation in 1972 of two new and apparently superfluid phases of liquid helium three below 3 mK thus, at a stroke, doubling the number of superfluids known in nature. The authors are to be commended for having managed to slip a page on this topic into the last chapter but, even so, the pace of advance has been such that already the passage strikes one as being principally of historical interest.

Vigorously and concisely written, the volume is lavishly adorned with diagrams and graphs of relevant experimental results, many of which will look comfortably familiar to workers in the field, but a significant number of which have not previously appeared within the pages of any textbook. There is a selection of problems at the end of each chapter except the last. In addition to references at the conclusion of each chapter, there is a select bibliography at the end of the book listing most of the standard texts and laced with descriptive comments, a number of which are amusingly barbed.

Thus in spite of one or two deficiencies, there is so much to be said on the positive side that I, for one, will strongly be recommending my graduate students and, indeed, some of my colleagues to read this book.

P. V. E. MCCLINTOCK

Analysis of rocks

Laboratory Handbook of Petrographic Techniques. By Charles S. Hutchinson. Pp. xxvii+527. (Wiley: New York and London, 1974.) £10.60.

THE title implies a rather unexciting prospect for the reader. Petrography is a vital part of petrology, and few scientists concerned with the genesis of rock complexes can afford to neglect the textures and mineral associations within a rock sample. Even so, it is the petrological analogue of anatomy in medicine, and has recently been in danger of neglect during the race by earth scientists to embrace geophysics and geochemistry as providing the main information for understanding the evolution of the Earth. Petrographic analysis is not essential for all geological studies, but there are few in which it does not demand an important role.

Some petrographic techniques have remained essentially unchanged throughout this century, such as polarised-light microscopy, mineral separation, and determination of specific gravity and refractive index. There have, however, been advances in method and this book presents an up-to-date survey of the whole field. The real advance made here, compared with earlier books on the subject, is the inclusion of sections on X-ray diffraction, differential thermal analysis and thermoluminescence measurements, and the expansion of the con-

cept of petrography by including geochemical analysis by X-ray fluorescence, atomic absorption, and other techniques for components that cannot be analysed by physical methods. An excellent chapter on "Display of Data" deals rigorously with norm calculations, variation diagrams for rocks and minerals, and the statistical analysis of data.

The book is designed as a laboratory manual, and step-by-step instructions are given for all the techniques, ranging from photomicrography to the determination of feldspar structural state, matrix corrections in XRF spectrometry, or the calculation of mineral formulae. The subjects are well illustrated, and the book makes interesting reading. The author has, in fact, been remarkably successful in bringing new life to an old subject, probably because he conveys the impression that each technique has been subjected to his critical usage, and because he makes lively quantitative recommendations that are difficult to fault.

There is some overlap with *Physical Methods in Determinative Mineralogy* (edit. by J. Zussman) and *Methods in Geochemistry* (edit. by A. A. Smales and L. R. Wager), although surprisingly he does not refer to the latter. Nevertheless, the emphasis of this book is different and there is no real equivalent for a research student or advanced-level undergraduate specialising in petrology. It will save supervisors and tutors many hours of routine instruction, and may extend the life expectancy of much laboratory equipment.

My major criticism of this otherwise excellent book is the inclusion of 68 pages on X-ray fluorescence spectrometry which, like electron probe analysis, is dealt with in specialist texts. The author omitted electron probe techniques partly for that reason, whereas I would view them as vital to petrographic analysis. In contrast, the section on reflected-light microscopy is absurdly brief (about 6 pages) despite its vital role in petrography and the difficulties encountered by students.

Other notable omissions, in the context of the author's aims, are single-crystal X-ray diffraction (for example anorthite structural state), petrofabric analysis, factor analysis, electron microscopy and Mössbauer spectroscopy. Some attention to problems of zoned crystals (refractive index section) and modal analysis (for example use of reflected light), and to mineral examples (eight pages for garnets versus one third of a page for clinopyroxenes, under X-ray diffraction) are also needed, in any revised edition.

G. M. BROWN

Please take note

Effective Technical Writing and Speaking. By Barry T. Turner. Pp. xi+206. (Business Books: London, 1974.) £4.75.

THE Professor of Industrial Management at the University of Newcastle has aimed his book at engineers and scientists in industry; there are chapters on how to write specifications, sales literature, contracts, patents and handbooks. But the sections on writing articles and reports, on organising meetings, on giving talks and how best to illustrate them, will interest academics as well.

Professor Turner's advice on all these topics is sensible and helpful. Some of it might even seem obvious, were it not for the incomprehensible handbooks, the turgid and jargon-filled articles, and the ill-prepared lectures with illegible slides, that we are all unhappily familiar with.

We are urged to avoid technical jargon in our writing; to use short sentences and short Anglo-Saxon words; and to approximate to con-

versational speech. It helps, we are told, to "write in haste, polish at leisure". A few false grammatical idols are toppled: "... grammar is common speech formulated—usage is the only test" (Somerset Maugham). The Professor disapproves of foreign phrases, which is unfortunate because "*le mot juste*" appears on page 2—professorial licence perhaps.

There are a few misprints in the book, some of which are amusing: ("The medium is the massage"; MacLuhan). More serious is an error in a table on SI units, in which kilo, mega and giga imply factors of 10^6 , 10^9 , and 10^{12} , respectively. Recommended reference books do not include a dictionary of quotations, which is perhaps why George Eliot is misquoted. And in a book devoted to better writing the use of "disinterested" to mean "uninterested" is regrettable.

In general, however, Professor Turner's book makes useful reading for anyone speaking or writing on technical subjects. It would be a profitable addition to any library.

JOHN WALKER

Revolution in physics

The History of Quantum Theory. By Friedrich Hund. Translated by Gordon Reece. Pp. 260. (Harrap: London, March 1974.) £6.10.

THE main part of this book is a blow-by-blow account of the development of quantum theory in about the first 28 years of the present century, from Planck's introduction of light quanta through the 'old quantum theory' up to the formulation of quantum mechanics and wave mechanics culminating in Dirac's relativistic equation for the electron. The whole of existing basic quantum theory may be said to have been produced in about the three years 1925-8. The school of Max Born in Göttingen, including Heisenberg, Jordan and Pauli played a leading part at that time and Professor Hund was himself a prominent member. So here we have a first-hand account of all that was achieved in those heroic times of phenomenal activity, as well as the story of what had led up to this. It would be almost impossible for anyone ever again to provide such a survey, and the history of science will owe a great deal to Professor Hund for giving it.

The important thing is that this account has been written. It would, however, have been interesting to know more about the history of the history. The brief foreword is undated and there are no bibliographical particulars whatever of the original German publication save for its title: *Geschichte der Quantentheorie*. There are a few references to relevant items in the literature after about 1930 up to the present, but the text could have been written any time in that interval.

Within its set design, the history is comprehensive and exceedingly well digested. It is clear and it is also very terse. Its value as a historical document is all the greater for these reasons. But they almost inevitably eliminate the excitement and romance. Before the first World War there were tremendously revolutionary ideas about atoms, photons, space and time. After that there was an interlude when all kinds of things about atomic spectra—transition rules, line intensities and the like—were got with extreme ingenuity by using a wierd combination of classical and quantum physics with astonishing elaboration and success but with no new basic idea. Then suddenly quantum mechanics in its several varieties came into bloom and fruition; its doing so seemed curiously inevitable though the forms were wholly unexpected. The facts are all here on permanent record; however, there has never been a time like that in the history of physical thought and one doubts whether the reader in a different epoch will be able

to re-create from the bare facts the stimulus of living through it.

Also I am convinced that no historian has yet explained how there came to be such a rush of discovery by so many scientists in so many places all at the same time. All previous fundamental advances seem to have been largely the work of one outstanding individual at a time—one may think of Newton, Maxwell, Rutherford, Einstein, Bohr as examples—but in 1925-8 fundamental contributions came from maybe 20 individuals of comparable stature (no doubt some more comparable than others), several of them only half the age of the others, working in some 10 or so different institutions. Hund does

indeed indicate these aspects in a brief note on "Centres of Research" at the end of his book; it contains a most interesting diagram on page 251 showing the times when a number of the leading workers made their contributions, but this is presented without explanation or comment. Possibly someone else, working from all this material so ably marshalled by Professor Hund, will some day succeed in revealing the underlying historical causes of the phenomenon.

The book is expensive for its number of pages, but perhaps not for the concentration of information they convey. Gordon Reece's translation is outstanding. W. H. MCREA

Some useful ammunition for worried ornithologists

The Seabirds of Britain and Ireland. By Stanley Cramp, W. R. P. Bourne and David Saunders. Pp. 287. (Collins: London, 1974.) £3.50.

HERE is a classic for the last-page-first reader since the final appendix clearly illustrates both the origins and the uses of this book. In this appendix figures are given which demonstrate the fate of puffins at various breeding colonies in Britain and Ireland during the last 100 years or so. The immense colonies on so many of Britain's islands at the turn of the century are all seriously depleted or even deserted; the 20,000 birds on Puffin Island, County Kerry in 1965 had declined to 3,700 five years later. Not that all is gloom with Britain's seabirds. As this book shows, gannet, kittiwakes and fulmars have all had population expansions, even explosions, in recent decades. Not only are the causes of these fluctuations unclear but all too often their extent is little more than guesswork. In order to build a sound basis for assessing future fluctuations, the Seabird Group, founded in 1965, took upon itself the onerous task of making as full as possible a census of the breeding seabirds of Great Britain and Ireland. After a pilot study in the year of the Torrey Canyon disaster, the full survey was carried out in 1969-70. "Operation Seafarer" as it was known, yielded several volumes of detailed results. The essence of these forms the basis of this book.

Twenty-four regularly breeding species are considered under the title of seabirds although such thoroughly maritime birds as the eider duck and the oyster catcher are arbitrarily excluded. The bulk of the text is composed of a section devoted to each species and covering their identification, breeding, world distribution and past and present status in the areas surveyed, together with a note on the

census method used for each species except, inexplicably, any of the gulls. These sections are sound and well referenced. Only the identification details, even in conjunction with the rather mean drawings and photographs, leave something to be desired; but they are probably superfluous anyway.

The rest of the book consists of three chapters on seabirds—dealing with their biology, the threats to their existence and their present numbers and changing fortunes. These make informative, if not always elegant, reading. At the back of the book are placed a series of maps and tables. The former, one per species, show the locality, number and size of the breeding colonies discovered in Operation Seafarer. Crispin Fisher is to be congratulated on presenting in them a large deal of information in a masterly way. The tables are an inconsistent set, sometimes supplementing the data in the maps, sometimes duplicating it.

Much of the information in the book is unique, all will be valuable. It is perhaps a pity that room could not be found for a more general chapter on bird population changes covering at least Europe and including inland as well as seabirds. It would also have been fascinating to know more about the organisation and logistics of the survey. We are told something about the census methods, which ranged from the smelling out of storm petrels' nesting sites to aerial photography of gannet colonies. But exactly how the hundreds of intrepid observers worked we never find out.

Despite some deficiencies this book should appeal to all who have more than just a passing interest in birds. As the sides line up for forthcoming battles over North Sea oil landing sites and other coastal attacks, it will be a source of much useful ammunition.

PETER NEWMARK

Culture and cognition

The Cultural Context of Learning and Thinking: An Exploration in Experimental Anthropology. By M. Cole, J. Gay, J. A. Glick and D. W. Sharp. Pp. xx+304. (Tavistock: London, June 1974.) £1.50.

THIS book is an important attack on the problem of the relation between culture and cognition. Too often in the past psychologists have travelled the world with a kit of psychometric tests, showing that on most tests "primitive" people obtain very low scores. They have concluded either that these people are innately less intelligent than those in western society, or that aspects of their environment have hindered their intellectual development. In both cases, what is posited is an intellectual deficit, a failure to develop the most powerful cognitive structures. Recently, however, a number of psychologists have argued that cognitive skills of the same order develop in all human groups; what differs between groups is the contexts in which these skills are applied. In order to assess the skills of another culture it is necessary to understand the tasks it imposes on its members. This book describes an interdisciplinary study by anthropologists, linguists and psychologists of cognitive skills in one African society.

The authors started with an educational problem; the Kpelle children in Liberia experience great difficulty with western type mathematics. Yet in their daily lives the Kpelle use quantitative operations and argue skilfully and with logic. In order to resolve this paradox the authors developed a series of experiments that would throw light on the Kpelle concept system and their methods of learning and problem solving. They first attempted to describe the Kpelle classificatory categories, and then used them in their investigations of learning, remembering, and concept formation with groups of non-literate children and adults, and primary and secondary school children.

A brief account of one investigation will give the flavour of the work. Kpelle schoolchildren seem to learn by rote, without grasping the principle involved. The authors confirmed this observation experimentally; in classical learning tests the initial recall of the Kpelle was poor, and increases with practice were negligible. These deficits seemed to be related to their failure to impose any order or structure on the material to be learned. Performance was no better when objects were substituted for words. The authors sought ways of evoking efficient performance. Attempts to improve motivation by a variety of rewards were unsuccessful; however, when the task was structured, either by

indicating the categories involved, or by embedding the words in a story, recall dramatically improved. Only the high school students could impose structure on the learning material themselves.

From these and many other experiments, and from a study of Kpelle society, the authors conclude that the non-educated Kpelle is capable of concept-based learning, inference and logical thought, but that the situations in which he applies these skills are different and more restricted than those of his high school age mate. The findings raise many questions—for example, what learning experiences are crucial in order to develop transferability of skills—and this important book should stimulate more work by educationalists and psychologists.

BARBARA TIZARD

A theoretical view of life

Molecules and Life: An Introduction to Molecular Biology. By Mikhail V. Vol'kenshtein. Translated from the Russian by Serge N. Timasheff. Pp. xiii + 513. (Plenum: New York and London, 1974.) \$9.55.

MOLECULAR biologists at work may be compared to industrial archaeologists: pieces of the machinery are carefully retrieved, dusted and measured and parts of the mechanism are put together in the test tube and made to work. This sort of activity has been in full swing for fifteen years or more and has led to an impressive, though far from complete, understanding of what makes living things tick. In the background of this activity have hovered physicists and theoreticians who have been crucial in inventing and applying new methods of gathering information and, more peripherally, who have attempted to explain the properties of interesting biological components in terms of fundamental physical theory. The latter have often impressed and sometimes enlightened the less sophisticated biochemist but I would guess that they have rarely blazed pathways for molecular biology.

Academician Volkenshtein has made important contributions to our understanding of polymers and several other fields and has in recent years turned his interest to biology. One of the fruits of the latter interest is this volume—first published in the Soviet Union in 1965 and now available in revised form in English in a paperback edition. As the author says in his preface the book deals principally with the structure and function of nucleic acids and proteins but, throughout, the quest of the theoretician for regularities and generalisations is apparent. This is first manifest in the first chapter which is devoted to grand concepts such as statistical mechanics, irreversible thermodynamics

and information theory. These notions and their application to biology are explained with admirable clarity but one is left with the feeling that they are not of immediate relevance to the working molecular biologist in that they tend to disappear into abstraction. To take one example, an argument is presented in refutation of the hypothesis of the inheritance of acquired traits in terms of the proposition that no flow of information from somatic to sex cells has been detected. I am sure he is right but an experimental fact or so might have been more convincing. In similar vein, the text which is otherwise descriptive breaks into mathematical derivation and matrices and their Eigen values loom up from the pages. These may deter the biologist and it would be better to have a degree in physics or chemistry with some mathematical experience before embarking seriously on this book. But this is not meant as a criticism and more experienced workers will find much to interest and enlighten them in this book for topics not covered in similar works are given space; simple treatments of helix coil transition theory are hard to find and the sections on the genetic code and on polymer conformation are especially interesting.

Nevertheless, the graduate student entering the field for the first time and to whom I hesitate to recommend this book should beware of several errors and misleading passages. One of the more serious occurs in the chapter on protein structure. There, the idea is presented that the success that Perutz achieved in the X-ray crystallography of haemoglobin by using heavy atom derivatives had something to do with making the diffraction spots sharper and revealing the location of the hydrogen atoms, rather than being the crucial method of overcoming the phase problem that it was. In the chapter devoted to a cytological description of the living cell, no prominence is given to the distinction between prokaryotes and eukaryotes and the significance of the single sentence that says that bacteria do not have a visible nucleus is unclear.

Again, as is to be expected in a book first published in 1965, much recent work is not mentioned and hence there is little on ribosome structure, protein synthesis or the structure of tRNA, to mention just a few topics. In fact there are few references dated later than 1965 so to read this book in isolation would give a somewhat antiquated picture of the state of molecular biology. One would question the wisdom of issuing a paperback edition of a book first published nine years previously were it not for the distinctive flavour of the approach and the light it may throw on thinking on molecular biology in the Soviet Union.

E. G. RICHARDS